Potential Biological Control Agents for the European Green Crab, *Carcinus maenas*, in Australian Waters

by

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Portunion sp., an entoniscid isopod parasite of the shore crab *Cyclograpsus granulosus*

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DECLARATION AND AUTHORITY OF ACCESS

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ABSTRACT

Marine species are invading new ports and waterways threatening biological diversity and contributing to environmental changes which are difficult to reverse or remediate. The European green crab, *Carcinus maenas* (Linnaeus, 1758) is a successful invasive marine species which has spread from Europe to adversely impact the marine ecosystems of South Africa, and the continents of North and South America and Australia. Methods for controlling this crab are sought and biological control is one possible method.

This thesis examines the parasite fauna of native and introduced shore and near-shore crabs from the temperate coastlines of Victoria, Flinders Island and Tasmania, Australia, to search for potential biological control agents for the introduced pestiferous European green crab, *C. maenas*. Collections were made from the inter-tidal zone by hand and trap and from shallow sub-tidal (< 5m depth) zones using traps set from boats. The study area surveyed both established populations (first recorded > 100 yeas ago) and recently arrived populations (first recoded 12 years ago).
This survey revealed a number of potential biological control agents against *C. maenas,* including two species of trypanorhynch tapeworm, *Dollfusiella martini* (Beveridge, 1990) and *Trimacracanthus aetobatidis* (Robinson, 1959), and a new species of rhizocephalan. Field observations of high larval trypanorhynch loads in individual *C. maenas* showed evidence of gross pathology which was histologically studied and described. Physiological impairment of *C. maenas* was indirectly examined through digestive enzyme analysis of the parasitised digestive gland. Histology and digestive enzyme analysis revealed that *C. maenas* with high intensity

trypanorhynch infections suffered digestive gland damage and possibly impaired digestive enzyme function.

Taxonomic relatedness of native hosts with *C. maenas* was shown to be more important than ecological overlap for parasites to transfer to *C. maenas.* Consequently, attempts were made to cross-infect green crabs with a rhizocephalan found on the confamilial native crab, *Nectocarcinus integrifrons* (Latreille, 1825), in the laboratory. These attempts failed, however, the unnamed rhizocephalan was described and named as *Sacculina nectocarcini,* a congeneric to *Sacculina carcini* (Thompson, 1836) – a well documented parasitic castrator of *C. maenas* in its native European range.

An analysis of mitochondrial cytochrome oxidase I (COI) DNA of nominal *S. carcini,* parasitising three species of portunid crab, revealed that *S. carcini* is capable of parasitising at least two other species of portunid crab in addition to *C. maeanas.* The use of *S. carcini* as a biological control agent must be treated with caution. COI analysis proved to be a useful tool for resolving spatial heterogeneity of *S. carcini.*

In conclusion, larval trypanorhynch tapeworms offer some potential for control against *C. maeans.* However, the many unknown trophic links required to complete the lifecycles of these parasites will make field application unpredictably difficult. *S. nectocarcini* directly attacks its host, making field management as a biological control agent simpler and more effective. The host specificity of this parasite needs to be resolved to determine whether it will switch to *C. maenas.* The host specificity of

S. carcini, a rhizocephalan parasitising *C. maenas* in its native range, is too lax for it to be released safely in Australia.

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Marine Biological Invasions

The first human mediated marine biological invasions probably occurred shortly after the rise of the world's first seafaring civilizations. However, it was not until 500 years ago that the first major human assisted biological introductions occurred when European explorers re-established contacts between people of the new and old worlds (Baskin, 2002). It is highly likely that hull fouled wooden ships from this period transported new marine species to new locations around the world. It has been estimated, for example, that a wooden sailing ship in 1750 could have had a hull fouled with 120 marine organisms, with 30 more harbouring in dry ballast and the anchor chain (Carlton, 1999).

In modern times the dispersal of marine organisms into new environments has increased dramatically due to ships using large volumes of sea water for ballast (Occhipinti-Ambrogi and Savini, 2003). It has been estimated that presently, on any one day, up to 10, 000 different marine species are being transported around the globe in the ballast water tanks of ocean going vessels (Carlton, 1999), and the survival of marine organisms in modern ships is expected to increase as ship transit speeds rise, water quality of ballast tanks improves and ports are better environmentally managed (Bax *et al.*, 2003). Ballast water tanks are not the sole vector for modern marine biological invasions. Hull fouling, and unintentional releases from mariculture contribute to the majority of marine introductions with smaller contributions from semi-dry ballast and intentional releases (Bax, 2003, Siguan, 2003).

The impacts of these marine invasions on native biota are not fully understood and cannot be accurately predicted, as studies of marine invasions have been largely

inferential, rarely combining empirical field data and experimental results with extensive descriptive data (Grosholz and Ruiz, 1996). However, it is known that the rate at which foreign marine organisms are successfully establishing in new ports throughout the world is increasing (Ruiz *et al.*, 1997, Cohen and Carlton, 1998, Hewitt *et al.*, 1999, Wonham *et al.*, 2000). In turn, this is threatening biological diversity and contributing to environmental change (Elton, 1958, Carlton, 1999, Ruiz *et al.*, 2000). These changes can adversely affect human health, economic production in fisheries, aquaculture, tourism and marine infrastructure (Bax, 2003). The environmental, economic and social implications of marine biological invasions have only recently begun to receive close scrutiny (Bax *et al.*, 2001).

The invasion of the Great Lakes Region by the zebra mussel, *Dreissena polymorpha* (Pallas, 1771) in the late 1980's (Griffiths *et al.*, 1991) focused attention on the economic and environmental costs of introduced aquatic species. The zebra mussel began spreading from western Russia (Berkman *et al.*, 1998) throughout Europe in the early 1800's due to expanding canal networks (Morton, 1977) and was eventually introduced to the Great Lakes Region of North America via ballast water. The zebra mussel forms vast, dense colonies changing the physical nature of the substrates, producing ecological consequences for encrusting invertebrates and significant fouling costs for industry. It soon adapted attachment from hard surfaces to include soft sand, silt and mud sediments, further extending its range (Berkman, *et al.*, 1998). The rapid introduction of this species into new environments, has in all instances been due to the activities of man (Morton, 1977). The Great Lakes introduction highlighted the risk of spreading marine, estuarine and freshwater pests throughout the world via

uncontrolled ballast water discharge, a vector which has introduced numerous marine and estuarine pest species throughout the world (Carlton, 1999).

Like the zebra mussel, the Chinese mitten crab, *Eriocheir sinensis* (Milne Edwards, 1854), is an example of an aquatic pest which has been introduced via marine ballast water into estuarine and freshwater environments from its native range (Herborg *et al.,* 2003). The Chinese mitten crab's native range extends from Hong Kong to the border with North Korea and its introduced range now includes Continental Europe, Southern France, the west coast of the USA and the United Kingdom (Herborg *et al.,* 2003). This crab interferes with recreational and commercial fishing, causes river bank erosion by burrowing and may compete for food and habitat resources with other native species (Herborg *et al.*, 2003 and references cited therein). The European green crab, *Carcinus maenas* (Linnaeus, 1758) is an entirely marine species with a similarly expansive introduced world range. The green crab has been introduced to South Africa (Le Roux *et al.,* 1990) and the east and west coasts of the United States (Welch, 1968, Cohen *et al.,* 1995) with the most recent report confirming a predicted range extension to the Atlantic coast of Patagonia (Hidalgo *et al.,* 2005). A sibling species, *Carcinus aestuarii* (Nardo, 1847), is also found in Japan and South Africa (Geller *et al.*, 1997). Like the Chinese mitten crab, the green crab adversely affects commercial fishing (Walton *et al.,* 2002) and competes with native species (McDonald *et al.,* 2001).

Marine plants are a group well adapted to accidental introductions via ballast water on account of microscopic algal spores and single celled microalgae surviving in ballast water tanks. However, the marine alga, *Caulerpa taxifolia* (M.Vahl) C. Agardh, provides an interesting case of accidental introduction via the aquarium trade. This

hardy and attractive plant was accidentally introduced at a single location into the Mediterranean in 1984 from a public aquarium. By the year 2000, it had spread across the Mediterranean to cover 131 km^2 of benthos spanning six countries (Spain, France, Italy, Monaco, Croatia and Tunisia) (Meinesz *et al.,* 2001). Today this alga has displaced many native algal species and is likely to be changing the structure of native benthic communities (Piazzi *et al.,* 2001). The same invasive Mediterranean strain has also been identified in California (Jousson *et al.,* 2000) and Australia (Millar, 2004).

Marine Biological Invasions in Australia

Australia, being an island continent in the southern Indo-Pacific, has the great advantage of oceanic separation from other land masses along its temperate and sub-tropical coastline. Only the islands of Torres Strait, the remnants of the land bridge which joined Papua New Guinea to Australia between 18000 and 6000 years ago (Galloway and Löffler, 1974), provide a narrow corridor for possible plant and animal introductions to Australia's tropical coast (Chapman *et al.,* 2003). This geographical isolation has resulted in only sporadic marine introductions since European settlement, many of which can be attributed to accidental introductions associated with the commercial importation of live oysters (Willan, 1987). However, within the last 30 years or so, the number of marine invaders successfully establishing in Australian waters has increased considerably. Increased shipping trade comprised of larger and faster vessels have necessarily focussed attention on marine incursions delivered by ballast water, with some of the earlier records including: Asian mussels, *Musculista senhousia* (Benson in Cantor, 1842), (cf. Willan, 1987) toxic dinoflagellates (Hallegraeff and Bolch, 1991) and The North Pacific Seastar, *Asterias amurensis* (Lutken, 1871), (cf. Buttermore *et al.,* 1994).

Presently, over 200 introduced marine species and species of unknown origin (cryptogenic) have been estimated in Australian waters from port surveys, literature-searches and museum collections. Considering only 21 of Australia's 72 trading ports have been surveyed, this number may well be an underestimate (McEnnulty *et al.*, 2001). Of these introduced species, 12 have been officially listed as pests by the Australian Ballast Water Management Advisory Council and include the following: four species of toxic dinoflagellate [*Alexandrium catenella* (Whedon and Kofoid) Balech, 1985, *A.* minutum (Halim, 1960), *A. tamrense* (Balech, 1985), *Gymnodinium catenatum* (Graham, 1943)], one species of sea star (*A. amurensis*) one species of crab [*C. maenas*], three species of bivalve [*Corbula gibba* (Olivi, 1792), *Crassostrea gigas* (Thunberg, 1793), *M. senhousia*], one species of fan worm [*Sabella spallanzanii* (Gmelin, 1791)], one species of macroalgae [*Undaria pinnatifida* (Harvey) Suringar] and one species of bacterium (*Vibrio cholerae*) (see McEnnulty *et al.*, 2001). It should be noted that *V. cholerae* is considered autochthonous in Australian estuarine microbial communities (Reddacliff *et al.*, 1993) and is strictly, therefore, not an introduced species. This list of 12 may well expand as future introductions cannot be ruled out and changing environmental conditions may enable many of the dormant or nascent introduced species to become pestiferous. Presently, Australia's temperate coastline has eleven of the twelve species including the European green crab, *C. maenas*.

The European green crab was first introduced into Australia at the turn of the 20th century, probably by vessels dumping dry ballast in Port Phillip Bay (Fulton and Grant, 1902). Since then, the crab has appeared in every state of Australia except Queensland and the Northern Territory (Poore, 2004) and it was first discovered in

Tasmania in 1993 (Gardner et *al.*, 1994). *C. maenas* was recently discovered in Botany Bay, Sydney, describing a northerly range extension of nearly 300 km from Nangudga Lake, near Narooma, N.S.W. (Ahyong, 2005).

Various authors have reported the green crab to be a 'voracious' predator of infaunal invertebrates, competing with native crabs and possibly shore birds for food (Grosholz and Ruiz, 1995, MacKinnon, 1997, McDonald *et al.*, 2001) and reducing catches of clam fisheries (Lafferty and Kuris, 1996, Walton *et al.*, 2002). As a marine pest, *C. maenas* has been described as a worst case introduction (Kuris, 2002).

In the summer of 1996/97 an unprecedented number of green crabs were being caught in Georges Bay on the north eastern coast on Tasmania. Baited traps, set to fish overnight, were frequently full the following day with 200–300 crabs per trap (trap volume = 44 litres), with the highest single trap catch being 428 (Proctor and Thresher, 1997). Furthermore, the maximum size of *C. maenas* from Tasmanian waters was possibly the largest, compared with published figures for native and introduced populations of *C. maenas* worldwide (Proctor and Thresher, 1997). It is common for introduced species to attain larger sizes than conspecifics in their native range (Torchin, 2002). This rapid rise in abundance of *C. maenas* in Tasmania, from the initial discovery in 1993, was reported at an international workshop on the impacts and management of introduced populations of *C. maenas* (Thresher, 1997). The workshop considered both physical removal and biological control of *C. maenas*. The perceived benefits of physical removal included: immediately available technology, minimal damage to native species through the selective removal of targeted green crabs, and the possibility of a profitable return on green crabs, provided a market

could be found. Biological control in the marine environment was, at the time, a new idea which had arisen from modelling the impact of parasitic castrators and symbiotic egg predators on crustacean fisheries (Kuris and Lafferty, 1992) and developed as a theoretical basis for biological control (Lafferty and Kuris, 1996). The potential for biological control of the green crab was considered to be good based on the long history of study by European biologists of its many natural parasites and enemies.

Marine Biological Control

Biological control is the use of living organisms (biological control agents) to attack and control organisms which adversely affect human interests (pests). Pest organisms are generally non-natives that have been introduced into a region where they mal-adapt to the environment to cause ecological and economic damage. The aim of biological control, as its name suggests, is to control and regulate pest populations to acceptable levels rather than achieve eradication (Gause, 1969). The two key principles of safety and efficacy have been distilled from early, ad hoc, attempts in the late 18th and early 19th centuries to biologically control terrestrial pests. Safety primarily relates to host specificity (Lafferty and Kuris, 1996). A biological control agent must be host specific to ensure that only the targeted pest is attacked without unintended attacks on non-target species. It must also be efficacious, inflicting sufficient damage on the targeted host at the population level to ensure sustained damage and effective control. Specificity also confers efficacy by ensuring that the control agent closely tracks the density and distribution of the pest population thereby suppressing and regulating the pest.

Biological control can be divided into classical, neoclassical and augmentative forms. Classical biological control introduces a non-native control agent from the pest's native range to control it in its new range. Neoclassical biological control introduces a non-native control agent to control a native pest. Augmentative biological control enhances the population of native control agents to attack native or introduced pests (Secord, 2003).

There have been calls in the recent past for the biological control of marine pests (Wolfson *et al.*, 1978, Miller, 1985, Buttermore *et al.,* 1994). A framework for the implementation of biological control of marine pests was first designed nearly ten years ago based upon the established science of biological control for terrestrial pests in agriculture (Lafferty and Kuris, 1996), and marine biological control theory continues to be refined (Meinesz, 1999, Kuris and Lafferty, 2000). Lafferty and Kuris (1996) argue that a preceding 100 year history of terrestrial biological control offers lessons and insights to be modified and applied to the marine environment. The perceived benefits of biological control include: 1 successful natural enemies are able to follow pest populations and locate new populations as they eradicate others, 2 pests are less able to develop resistance as natural enemies are able to co-evolve with their hosts, and 3, well chosen agents offer long term or permanent control of pests.

There are a number of efforts throughout the world which are presently attempting to eradicate or control invasive marine pest outbreaks (see Thresher and Kuris, 2004). All of these efforts have concentrated on physical removal or the use of biocides which are reversible with minimal risk of long term unintended consequences to the environment or non-target organisms (Thresher and Kuris, 2004). These measures

have been successful where invasive species are rapidly detected at low population levels or are spatially confined or restricted (Bax, 1999, Culver and Kuris, 2000). However, the control of long established invasive pest species is unlikely to succeed by these methods where populations have become large and are spread over vast areas. Most discovered marine introductions are likely to be well established with large populations of reproductive individuals (Kuris, 2002). Marine biological control is an option worthy of careful consideration for established pests and this view was endorsed by international marine managers and scientists who considered a range of options for managing invasive marine species at a workshop convened to focus on technological approaches which were likely to be effective and publicly and politically acceptable (Thresher and Kuris, 2004).

While it is acknowledged that, to date, no biological control programs have been implemented for marine pests, there has been careful research into its potential, particularly in respect of controlling *C. maenas* with the parasitic rhizocephalan barnacle *Sacculina carcini* (Thompson, 1836) (cf. Goddard *et al.* 2001, Thresher *et al.* 2000, Murphy and Goggin, 2000) and the seastar *A. amurensis* with a ciliate *Orchitophyra stellarum* (see Goggin and Bouland, 1997).

The use of biological control in the marine environment has been met with caution (Simberloff and Stiling, 1996, Byrne, *et al.,* 1997, Murphy and Goggin, 2000) and qualified objection (Secord, 2003). Much of the concern centres on host specificity or safety. A biological control agent must only attack the intended pest target and not attack native fauna or commercially valuable hosts (e.g. crab and crayfish fisheries). Indirect effects on non-target organisms and the evolution of new host affinities over

ecological time are also of concern (Secord, 2003 and sources cited therein). The issues of safety and efficacy can not be resolved without further information derived from studies of taxonomy, pathogenicity and experimental studies on host specificity.

A number of studies have identified potential biological control agents against *C. maenas*, including for example: *Fecampia erythrocephala* (Giard, 1886) (cf. Kuris *et al.* 2002) *Carcinonemertes epialti* (cf. Torchin *et al.*, 1996) and *Sacculina carcini* (cf. Murphy and Goggin, 2000, Thresher *et al.*, 2000). In addition to finding natural enemies from the pest's endemic range (classical biological control) it is possible to look for control agents in the pest's invaded range which may switch from native hosts to the pest (augmentative biological control). Indeed, augmentative biological control forms part of an integrated method for marine biological control outlined by Lafferty and Kuris (1996) and has been proposed as a safer alternative to classical biological control (Secord, 2003).

The parasite fauna of Australia's shore crabs, from where potential host switching control agents for augmentative biological control might be derived, is not well studied or described and reflects the state of crisis of parasite taxonomy in Australia (Cribb, 2004). As a result, shore crab parasites from temperate Australian coastlines, reported in this thesis, were surveyed on a broad scale to identify possible control agents for 'enhancement' against *C. maenas*. From this survey three parasites, two species of trypanorhynch tapeworm and rhizocephalan barnacle, were identified as potential native control agents. I examine the taxonomy, pathogenicity and distribution of these parasites and I re-consider the safety of a parasitic barnacle, *S. carcini*, as an introduced biological control agent for *C. maenas*.

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CHAPTER 2

PARASITES OF NATIVE AND INTRODUCED CRABS CAUGHT OFF TASMANIA, FLINDERS ISLAND AND VICTORIA

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PARASITES OF NATIVE AND INTRODUCED CRABS FROM TASMANIA, FLINDERS ISLAND AND VICTORIA, AUSTRALIA

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ABSTRACT: We collected 1327 intertidal and shallow sub-tidal crabs of 19 species (8 families) from Port Phillip Bay and Western Port (Victoria), Flinders Island, the east coast of Tasmania, and a few sites on the north and west coasts of Tasmania. Two species of crab, *Carcinus maenas* (Linnaeus, 1758) and *Metacarcinus novaezealandiae* (Jacquinot, 1846) were exotic. At least 18 species of parasites, comprising cestodes, trematodes, nematodes, acanthocephalans, entoniscid isopods, rhizocephalans and nemerteans were recovered. Four unidentified species of trypanorhynch were found in native crabs. Other unidentified parasites included three species of entoniscid isopod, two species of nematode and two species of rhizocephalan. The introduced green crab *C. maenas* was parasitised by two species of trypanorhynch tapeworm. These were identified as *Trimacracanthus aetobatidis* (Robinson, 1959) and *Dollfusiella martini* (Beveridge, 1990). Parasite intensity and prevalence data are tabulated. Native crabs contained the most diverse assemblage of parasites. Two species of acanthocephalan were identified as *Corynosoma stanleyi* (Smales, 1986) and *Polymorphus sphaerocephalus* (Bremser in Rudolphi, 1819). No parasites were found in the introduced pie crust crab *M. novaezealandiae.* The most prevalent parasites were the trypanorhynch *D. martini,* which infected 100% of 14 *Nectocarcinus integrifrons* (Latreille, 1825) from Swan Bay in Port Phillip Bay, Victoria.

Key Words: crustacean parasites, *Carcinus maenas*, *Nectocarcinus integrifrons*, Grapsidae, Rhizocephala, Trypanorhyncha, *Sacculina*, biological control, Victoria, Flinders Island, Tasmania, Australia.

INTRODUCTION

An extensive survey of a regional assemblage of crabs for parasites would provide information on host specificity, the potential impact of the parasites on their host populations, and suggest the role of environmental variables on parasite abundance. It would also detect intermediate hosts for vertebrate parasites and perhaps reveal some unknown and interesting parasites. Such a survey has never been conducted.

The value of such a study was made urgent by the discovery of an abundant and expanding population of the European green crab, *Carcinus maenas* (Linnaeus, 1758), in Tasmania (Proctor and Thresher, 1997). On the coast of Tasmania, as elsewhere, this invasive crab appears to be a potentially serious pest with deleterious consequences for fisheries, mariculture (Le Roux *et al.*, 1990, Grosholz and Ruiz 1995, Walton 2002) and wildlife including shorebirds (Ruiz 1987). Native populations of the green crab from the Atlantic coast of Europe are adversely affected by an assemblage of parasites, particularly parasitic castrators (Torchin *et al.*, 2002). A meta-analysis of a wide range of introduced species showed that introduced populations have substantially fewer infectious natural enemies than do native populations (Torchin *et al.*, 2002). Hence, potential mitigation of the impact of introduced green crabs could include classical biological control using parasitic natural enemies from Europe or augmentative biological control using parasites of native crabs. To prepare for either approach, it is necessary to have background information on the available array of parasites of the native crab population. Such a study should focus on crabs that are taxonomically close to the green crab and on those that are ecologically similar to the green crab.

This study presents an extensive examination of the parasitofauna of a rich assemblage of mostly intertidal and nearshore crabs from Victoria, Flinders Island and Tasmania, Australia. This information will, 1) provide background data prior to a possible release of a biological control agent for the exotic green crab, 2) determine the native host sources for the list of parasites able to transfer to the green crab, 3) enable a comparison of the parasite burden of the green crab and the ecologically similar crabs that are its potential competitors, 4) indicate potential impact of native parasites on their native hosts, 5) investigate trophically transmitted parasites to indicate final predator-vertebrate hosts, and 6) reveal possible model systems for research on control of crab populations by parasites.

Shields (1992) provides the only comprehensive investigation of a taxonomically diverse assemblage of parasite from a crab host [*Portunus pelagicus* (Linnaeus 1758) in Moreton Bay, Queensland]. For some of the crabs in the present study there is information on trematodes (Smith 1981, Bell 1988), acanthocephalans (Pichelin *et al.*, 1998) and nemerteans (Bell and Hickman 1985).

Here we examine 1327 crabs of 19 species from 8 families. We report three types of infectious natural enemies: parasitic castrators, trophically transmitted parasites and symbiotic egg predators. The host use, geographic distribution, prevalence and intensities of these parasites are discussed, and for some, their impacts on their hosts were evident. We also report life history information for the several newly discovered species.

MATERIALS AND METHODS

Between 11 November 1996 and 30 March 1998, we collected specimens of 19 species of intertidal and sub-tidal crabs from 31 sites at Port Philip Bay and Western Port in Victoria, Flinders Island and Tasmania (fig. 1). The Grapsidae formed our largest family of our crab survey but representatives from seven other families were also included. Two species were not native to this region: *Carcinus maenas* and *M. novaezealandiae.*

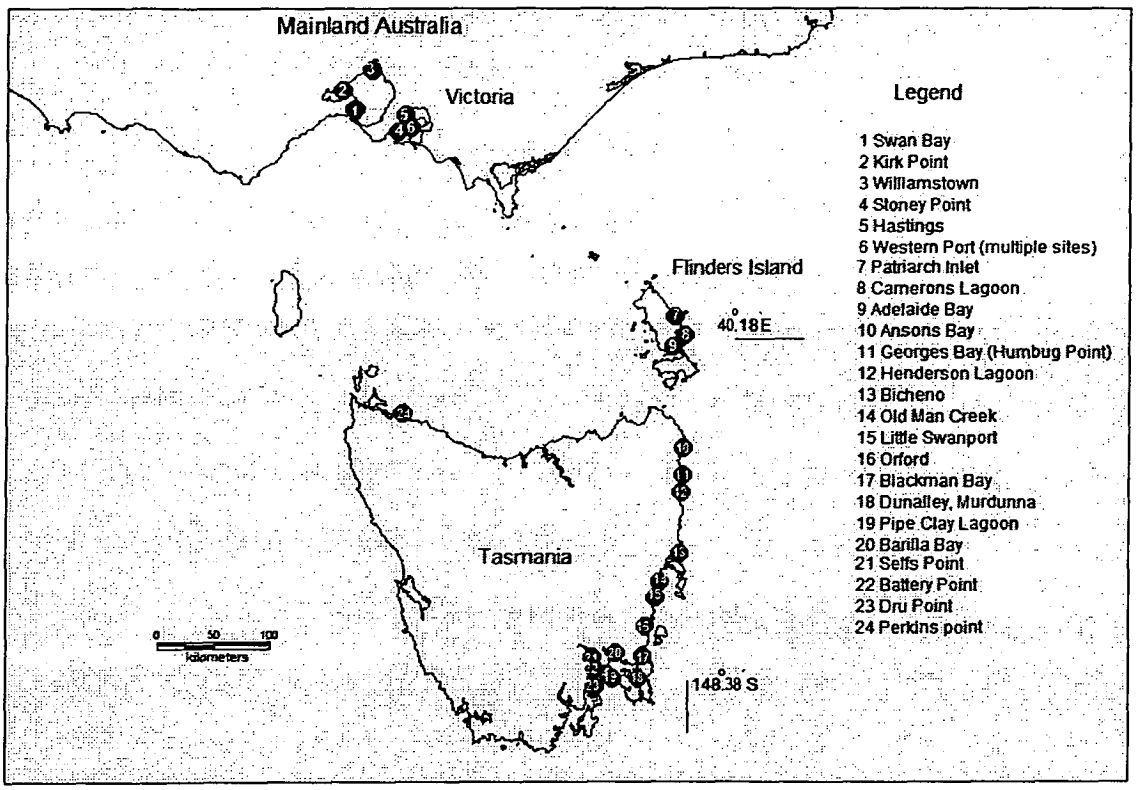

Figure 1. Crab sampling sites. Note the map scale prevents all sites being shown.

At each site, where possible, we collected at least 20 individuals, of at least 10 mm carapace width, of each crab species. Some additional large crabs were selected, if abundant, because most parasites accumulate in larger (older) individuals. The crabs were collected by hand or folding wire and plastic mesh traps. The substrate type was recorded at each site (Appendix A).

Crabs were identified to species with the aid of Hale (1927), Griffin and Yaldwyn (1971), Marine Research Group of Victoria (1984), and Smith, (1995). *Nectocarcinus* spp. were determined to species by direct comparison with specimens in the collections of the Tasmanian Natural History Museum.

The body surfaces of all crabs were examined for carcinonemerteans, rhizocephalans and nicothoid copepods, paying special attention to the limb axillae and the sternal-abdominal furrow. To expose the internal parasites, the carapace was gently lifted from its posterior connection to the thorax. Entoniscids were carefully separated from the host. The gills were examined for encysted carcinonemertean worms, rhizocephalan kentrogons and entoniscid cryptoniscus larvae and other epibionts. In ovigerous crabs, the egg mass was excised and teased apart to detect carcinonemerteans, turbellarians and nicothoid copepods. Discoloured or turbid blood was noted by examining the blood under the abdomen through the transparent arthrodial membrane alongside the hind-gut, and during dissection, in the body cavity and gills.

The digestive gland was examined under a dissecting microscope. In larger crabs (> 20 mm CW) about 0.5 g of the pyloric region of the digestive gland was removed and approximately halved. One half was squashed under a 22 x 50 mm coverslip on a microscope slide. Parasites seen in the squash were counted (and preserved for identification as necessary) to provide a quantitative assay across crab species and types of parasites. The remaining half was either squashed for examination or was teased apart to detect and recover parasites.

The general body cavity in the thorax and at the thoraco-abdominal juncture was examined and parasites removed. The presence of metacercaria in the thoracic ganglion and associated nerves was recorded but these parasites were not counted. The sides of the cardiac stomach and the buccal musculature were examined as were the paired digestive diverticulae. All metazoan parasites were counted other than digenean metacercariae (whose intensity was assayed in squashes of the digestive glands).

A 0.2 g squash of ovarian tissue of all crabs with a ripe or ripening ovary was examined. Digestive gland and ovarian squashes were not conducted for crabs collected in Western Port [except *N. integrifrons* and *Paragrapsus laevis* (Dana, 1852)] due to time constraints.

For rhizocephalans, the following developmental states were recorded: interna only, virgin externa, immature externa (ovary ripening, no eggs in mantle cavity), early reproductive externa (eggs without eyespots in mantle cavity), late reproductive externa (eyed eggs or nauplii in the mantle cavity), scar (from detached externa). Virgin externas were examined for the presence of presumptive male cyprid larvae attached to the mantle cavity pore. Entoniscid developmental stages 1–12 were recorded as per Kuris *et al.* (1980). Carcinonemertean life-cycle stages (juvenile, adult, regressed adult) were recognised as in Kuris (1993) and Bell and Hickman (1985). Acanthocephalan larvae were staged as acanthellae or cystacanths. The few dead parasites were counted and recorded.

Representative parasites were preserved for taxonomic identification and submitted as voucher specimens to the Tasmanian Museum and the South Australian Museum.

Digenean trematode metacercariae were identified with the aid of Bell (1988) and Smith (1981, 1983). Tapeworms were identified by Ian Beveridge, University of Melbourne with reference to Beveridge and Campbell (1987) and Beveridge (1990). Nematodes of the genus *Proleptus* were identified by George Poinar (Oregon State University) and acanthocephalans by Sylvie Pichelin (South Australia Museum) (see Pichelin *et al.,* 1998), verified with the aid of Smales (1986).

RESULTS

In all, 1327 individuals from 19 crab species were examined for parasites (Table 1).

Table 1. Crab species examined for parasites

Family	Species	*n*
Portunidae	*Carcinus maenas*	262
	Nectocarcinus integrifrons	258
	Nectocarcinus tuberculosus	12
	Ovalipes australiensis	50
Cancridae	*Metacarcinus novaezealandiae*	20
Grapsidae	*Cyclograpsus granulosus*	109
	Paragrapsus gaimardii	132
	Paragrapus quadridentatus	31
	Paragrapsus laevis	19
	Leptograpsus variegatus	5
	Brachynotus spinosus	55
	Helograpus haswellianus	31
Hymenosomatidae	*Halicarcinus* sp.	2
Leucosiidae	*Bellidilia laevis*	321
Majidae	*Notomithrax ursus*	6
	Naxia aurita	2
Xanthidae	*Pilumnopeus serratifrons*	2
	Psuedocarcinus gigas	7
Mictyridae	*Mictyris platycheles*	3
Total		1327

Two species are introduced: *C. maenas* and *M. novaezealandiae* (native to Europe and New Zealand respectively). Overall, 2 native species were parasitised by rhizocephalans, 5 by entoniscid isopods, 2 by carcinonemerteans, 4 by larval digeneans, 7 by larval cestodes, 3 by larval nematodes and 6 by larval acanthocephalans. Although no systematic investigation was made for microbial infections, we did recover bacterial infections (*Vibrio* sp.) in one species and gregarines in two species. A complete list of parasites and their hosts is presented in Table 2.

Table 2. The prevalence and intensity of parasites infecting their crab hosts

Host	Parasite	Registration Number	Sampling Location	*n*	Prevalence (%)	Intensity (mean)
Carcinus maenas	*Dollfusiella martini*		Kirk Pt. Vic.	14	35.7	7.6
			Stoney Pt., Vic.	16	12.5	1.5
			Stoney Pt., Vic.	28	10.7	1.3
			Sandstone I., Vic.	45	4.4	1
		K1752 (TM)	Swan Bay, Vic.	70	84	8.8
			Adelaide Bay, Flinders I.	5	60	13.6
	Trimacracanthus aetobatidis	K1751(TM)	Swan Bay, Vic.	58	48	2.2
			Adelaide Bay, Flinders I.	5	20	1

Host	Parasite	Registration Number	Sampling Location	*n*	Prevalence (%)	Intensity (mean)
			Ansons Bay, Tas.*	19	15.8	3.3
			Little Swanport, Tas.	20	10	1.5
	Proleptus sp.		Swan Bay, Vic.	70	1.4	3
Nectocarcinus integrifrons	*Sacculina* sp.	G5467(TM)	Stoney Pt., Vic.	58	32.8	
			Sandstone I., Vic.	24	58.3	
			Tortoise Head., Vic.	15	13.3	1
	Portunion sp. A		Sandstone I., Vic.	23	4.3	1
			Humbug Pt., Tas.	16	6.3	1
		G4345(TM)	Stieglitz Tas.	17	23.5	1.5
	Dollfusiella martini		Swan Bay, Vic.	14	100	33.3
			Stoney Pt., Vic.	33	48.5	3
			Sandstone I., Vic.	24	45.8	4.5

Host	Parasite	Registration Number	Sampling Location	*n*	Prevalence (%)	Intensity (mean)
			Sandstone I., Vic.	23	30.4	2.4
			Humbug Pt., Tas.	16	6.3	2
	Trimacracanthus aetobatidis		Swan Bay, Vic.	14	71.4	2.8
			Humbug Pt., Tas.	16	12.5	1
	Prochristianella moorae		Stoney Pt. Vic.	33	3	1
	Proleptus sp.		Swan Bay, Vic.	14	6	2
Cyclograpsus granulosus	*Portunion* sp. B		Adelaide Bay,Flinders I.	8	12.5	2
			Dru Pt., Tas.	9	33.3	1
			Old Man Ck., Tas.	35	20	1.1
		G4354(TM)	Battery Pt., Tas.	20	20	1
	Microphallus sp.		Dunalley, Tas.	22	9.1	4

Host	Parasite	Registration Number	Sampling Location	*n*	Prevalence (%)	Intensity (mean)
	Dollfusiella martini		Adelaide Bay,Flinders I.	8	37.5	1.3
			Dunalley, Tas.	22	9.1	1
			Murdunna, Tas.	15	6.7	1
			Murdunna, Tas.	15	6.7	1
	Corynosoma stanleyi	27991, 27992 (SAM)	Dunalley, Tas.	22	59	5.5
	Polymorphus sphaerocephalus		Old Man, Ck., Tas.	35	5.7	1.5
			Dunalley, Tas.	22	9	1.5
			Battery, Point Tas.	20	20	5
Paragrapus gaimardii	*Portunion* sp.B	G4350(TM)	Dunalley, Tas.	35	14.3	

Host	Parasite	Registration Number	Sampling Location	*n*	Prevalence (%)	Intensity (mean)
			Murdunna, Tas.	31	3.2	
			Battery Point, Tas.	36	2.8	
			Perkins Point, Tas.	5	20	
			Dru Point, Tas.	13	7.7	
	Microphallus sp.		Barilla Bay, Tas.	6	33.3	3
			Dunalley, Tas.	35	88.6	0.9
			Adelaide Bay, Flinders I.	5	20	6.5
	Maritrema eriolae		Murdunna, Tas.	31	41.2	2.1
	Dollfusiella martini		Orford, Tas.	6	16.7	1
			Barilla, Bay Tas.	6	83.3	6.4
			Dunalley, Tas.	35	11.4	3.2
		31345, 31347 (SAM)	Murdunna, Tas.	31	51.6	2.1

Host	Parasite	Registration Number	Sampling Location	*n*	Prevalence (%)	Intensity (mean)
	Trimacracanthus aetobatidis	31349 (SAM)	Dunalley, Tas.	35	2.9	1
			Adelaide Bay, Flinders I.	5	20	1
			Murdunna, Tas.	31	6.4	1
	Tetraphyllidea	31346 (SAM)	Barilla Bay, Tas.	6	33.3	5.5
	Polymorphus sphaerocephalus	27993 28007 (SAM)	Battery Pt., Tas.	35	42.9	4.1
			Orford, Tas.	6	50	3
			Barilla Bay, Tas.	6	33.3	2.5
			Murdunna, Tas.	31	3.2	1
			Adelaide Bay, Flinders I.	5	40	1
			Dunalley, Tas.	35	11.4	1.3

Host	Parasite	Registration Number	Sampling Location	*n*	Prevalence (%)	Intensity (mean)
	Corynosoma stanleyi		Dunalley, Tas.	35	2.9	1
			Adelaide Bay, Flinders I.	5	20	1
Paragrapsus quadridentatus	*Maritrema eriolae*		Dunalley, Tas.	16	68.8	2.9
			Perkins Pt., Tas.	7	42.9	1.7
	Polymorphus sphaerocephalus		Battery Pt., Tas.	8	12.5	1
			Dunalley, Tas.	16	6.3	1
	Corynosoma stanleyi		Dunalley, Tas.	7	12.5	1
	Dollfusiella martini		Perkins Pt., Tas.	7	14.3	1
			Dunalley, Tas.	16	12.5	2
Paragrapsus laevis	*Dollfusiella martini*		Adelaide Bay, Flinders I.	5	40	1.7
			Queenscliff, Vic.	3	66.7	1

Host	Parasite	Registration Number	Sampling Location	*n*	Prevalence (%)	Intensity (mean)
	Polymorphus sphaerocephalus	27993–28007 (SAM)	Hanns Pt. Vic.	11	72.7	1
Leptograpsus variegatus	*Dollfusiella martini*		Adelaide Bay, Flinders I.	5	60	1.7
			Dunalley, Tas.	14	21.4	1
Brachynotus spinosus	*Portunion* sp. C		Murdunna, Tas.	16	6.3	1
	Dollfusiella martini		Murdunna, Tas.	16	12.5	1
			Dunalley, Tas.	13	23.1	1
			Adelaide Bay, Flinders I.	5	40	1.7
	Polymorphus sphaerocephalus		Dunalley, Tas.	13	15.4	1.5
			Adelaide Bay, Flinders I.	5	40	3

Host	Parasite	Registration Number	Sampling Location	*n*	Prevalence (%)	Intensity (mean)
			Perkins Pt., Tas.	7	57.1	1
Helograpsus haswellianus	*Portunion* sp. D		Murdunna, Tas.	3	33.3	1
			Patriarchs Inlet, Flinders I.	17	11.5	1
			Henderson Lagoon, Tas.	11	9	1
	Gynaecotyla hickmani		Patriarchs Inlet, Flinders I.	17	5.8	6
	Maritrema erioliae			17	18	1.3
Bellidilia laevis	Undescribed rhizocephalan		Barilla Bay, Tas.	19 5	16.4	1
			Chilcott Rocks, Vic.	67	9	1
	Carcinonemertes sp.	K1749 (TM)	Barilla Bay, Tas.	19	4	1

Host	Parasite	Registration Number	Sampling Location	*n*	Prevalence (%)	Intensity (mean)
			Pipe Clay Lagoon, Tas.	25	6	1.3
	Maritrema erioliae		Barilla Bay, Tas.	34	17.6	4
			Pipe Clay Lagoon Tas.	25	56	6.2
	Gynaecotyla hickmani		Barilla Bay, Tas.	34	23.5	31.3
	Dollfusiella martini		Adelaide Bay, Flinders I.	18	28	1.8
Pseudocarcinus gigas	Nemertea			7		1.8

* Key to abbreviations:

Vic. = Victoria

Tas. = Tasmania

Flinders I. = Flinders Island

SAM = South Australian Museum

TM = Tasmanian Museum and Art Gallery

Introduced species

Green crab, *Carcinus maenas* (fam. Portunidae): Three species of helminths were recovered from the green crabs: two larval trypanorhynch tapeworms, *Dollfusiella martini* (Beveridge, 1990) and *Trimacracantus aetobatidis* (Robinson, 1959), and a larval nematode, *Proleptus* sp.

D. martini was the most prevalent parasite. The greatest prevalence, 84%, was recorded in Swan Bay, Victoria, while the highest average intensity was 23 worms per infected crab on Flinders Island. *D. martini* was not recovered from green crab populations in Tasmania. The encysted worms were found in the digestive gland. These white worms were small: 1 mm long encysted, 4 mm long excysted and extended (2 mm scolex and neck; 2 mm blastocyst). The digestive glands of heavily infected individuals from Swan Bay were white, rather than the bright orange of uninfected or lightly infected *C. maenas*, and the digestive gland tubules next to these small white worms were sometimes shrivelled and melanised.

Trimacracanthus aetobatidis was recovered from green crabs in Victoria, and Flinders Island and the eastern coast of Tasmania. It was less common than *D. martini*: reaching a maximum prevalence of 48% and maximum mean intensities of only 2.2 worms per host. The encysted worms were pink from the pigment of the blastocyst and the encysted stage was 3–4 mm long. Excysted, these worms extended to 10 mm (5 mm scolex plus neck; 5 mm blastocyst). The cyst wall was quite tough and resisted rupturing with a probe.

The large, pink oblate spheroid plerocercoid cysts were almost exclusively recovered from the subcardiac haemocoel pocket anterior to the third thoracic arthrophragm. They displaced most or all of the digestive gland that is normally present in the subcardiac pocket. The encysted parasites were directly above the nerves that lead from the thoracic ganglion to the claws, mouthparts and other cephalic structures. Since the exoskeleton and the cardiac stomach spatially constrict this area, only two to three encysted trypanorhynchs could easily be contained at this location. When more than three were recovered (up to six were sometimes present), they were smaller than the worms in low intensity infections.

The third helminth detected (*Proleptus* sp.) was found only in one crab from Queenscliff. It was infected with 3 larval nematodes encysted in the digestive gland and the haemocoel.

Pie Crust crab, *Metacarcinus novaezealandiae* (Jacquinot, 1846) (fam. Cancridae): Of the 21 *M. novaezealandiae* collected from two sites in south-eastern Tasmania, all were free from parasites. However, three of 21 crabs from Littleton, South Island, New Zealand were heavily parasitised by a neurotropic metacercaria (Kuris, unpubl. obs.).

Native crabs

Surf crab, *Ovalipes australiensis* (Stephenson and Rees, 1968) (fam. Portunidae): No parasites were detected in *O. australiensis* (27 examined from great Oyster Bay in southeastern Tasmania and 23 from Western Port, Victoria).

Red rock crab, *Nectocarcinus integrifrons* (Latreille, 1825 (fam. Portunidae): An undescribed species of rhizocephalan barnacle, *Sacculina* sp., had been previously discovered at Western Port, G. Ruiz, Smithsonian Environmental Research Center (SERC) (G. Ruiz, pers. comm.). Prevalences of this parasite ranged from 13% to 58% at three sites in Western Port (Sandstone Island, Stoney Point, Tortoise Head) but they were not found at four other sites in Western Port (Merricks Beach, Somers, Hastings Bight, Chilcott Rocks) or at any other sites.

The externa of this rhizocephalan is yellow, changing to grey as the eggs ripen and the mantle cavity becomes filled with nauplii. The interna is yellow-orange, large and elastic when probed. Of the 35 parasitised specimens, 60% had an externa. The remaining 40% of infections were identified with interna by dissection. No multiple infections were collected, although two double infections were found from this area in a later investigation, R. Gurney (unpubl. obs.). One virgin externa and one specimen with a scar where an externa had been attached were collected. The interna in the latter crab was extensive and appeared to be alive. No infected crab had a visible gonad. All male crabs with externas were feminised: their abdomens were wider than uninfected males.

An undescribed entoniscid isopod (cf. *Portunion* sp. A) was recovered from *N. integrifrons* at three of the eleven sites. Prevalence was highest at Steiglitz, in Georges Bay, Tasmania (23.5%), but lower at Humbug Point, also in Georges Bay (6.3%) and at Sandstone Island, Victoria (4.2%). Most infections were single infections, but one crab with three juvenile female parasites was collected. All female parasites had dwarf males and presumptive male cryptoniscus larvae within the host response sheath. The ovaries of the parasite were salmon pink in colour. Infected female crabs lacked ovaries, but the sperm ducts of male crabs were filled with sperm.

The most abundant trophically transmitted parasite was the trypanorhynch *D. martini*. It reached a prevalence of 100% and a mean intensity of 33.3 at Queenscliff, and was also common in Western Port. It was not recovered from *N. integrifrons* collected from Flinders Island or Tasmania. In the Queenscliff sample, mean intensity was 23 per crab, in crabs less than 35 mm carapace width and 39 per crab in larger individuals.

The digestive gland of parasitised individuals contained numerous necrotic tubules and the white colour of the digestive glands appeared to be due to amoebocytic interactions near the larval *D. martini* (detailed in Gurney *et al.*, 2004). In some of the crabs doubly infected with *D. martini* and *Sacculina* sp., the interna roots of the latter appeared to be particularly dense around the encysted tapeworm larvae.

The trypanorhynch *T. aetobatidis*, was also common at Queenscliff, being found in 67% of crabs less than 35 mm and 80% of crabs greater than 35 mm. Mean intensity

was 2.8 worms per infected host. It was rare elsewhere. Almost all the encysted worms (93%) were recovered from the subcardiac pocket of the haemocoel, largely displacing the part of the digestive gland normally found there. When more than three encysted worms were present in the sub-cardiac pocket, they were smaller than usual. Up to six were occasionally found within the pocket.

A single metacestode of *Prochristianella mooreae* was taken from an *N. integrifrons* captured in Western Port. This worm was encysted in the haemocoel of the host. It was a slender worm, 12 mm long, with a diffuse red pigment extending along the region of the neck with the muscular bulbs. The posterior tip of the worm was pointed and had an outer jacket of golden yellow cells. These faded and were sloughed immediately when the worm was immersed in fresh water.

Larval nematodes of the genus *Proleptus* were recovered from Queenscliff (prevalence 14%; mean intensity 6) and rarely from Western Port (2 infected crabs). Another crab from Western Port had a high intensity infection (45) of an unidentified species of nematode (not *Proleptus*). They were not encapsulated, lying free in the haemocoel.

A single acanthocephalan cystacanth of *Polymorphus (Profillicolis) sphaerocephalus* (Bremser in Rudolphi, 1819) was collected from the haemocoel of one crab at Stoney Point, Western Port. A specimen of the lepidomorph barnacle, *Octolasmis* sp., was observed in the gill chamber of one crab from Western Port.

Velvet crab, *Nectocarcinus tuberculosus* (Milne Edwards, 1860) (fam. Portunidae): Twelve *N. tuberculosus* from Port Davey in southwestern Tasmania were examined. No parasites were detected.

Cyclograpsus granulosus (Milne Edwards, 1853) (fam. Grapsidae): Only samples from Flinders Island and Tasmania were examined. An undescribed entoniscid isopod (cf. *Portunion* sp. B) occurred at Adelaide Bay (Flinders Island) and Dru Point, Battery Point and Old Man Creek (Tasmania). Prevalences ranged from 12% to 33%, and mean intensities varied between one and two. Female parasites were in the haemocoel. The digestive gland in parasitised crabs was noticeably smaller than in uninfected crabs, while the ovary was obliterated.

A few microphallid trematode metacercariae were detected encysted over the thoracic ganglia of 9% of the crabs from Dunalley at the western end of the Denison Canal. Based on their size, shape, site and host, these were probably *Microphallus paragrapsi* (Smith, 1983).

Dolfusiella martini was recovered from 38% of the crabs from Adelaide Bay (Flinders Island) and less commonly from Dunalley and Murdunna in south-eastern Tasmania. The worms from the south-eastern Tasmanian sites were smaller (2–3 mm, extended length), and the blastocyst was shorter (< 1 mm), than the specimens from Flinders Island and the abundant material referable to *D. martini* in the portunids (2 mm extended length, 2 mm blastocyst).

One crab at Murdunna was infected with a small larval nematode that lacked the cephalic collar of *Proleptus*. It was encysted in the digestive gland.

The bird acanthocephalan *P. sphaerocephalus* was a common parasite of *C. granulosus*, reaching 100% prevalence at a mean intensity of 21.5 worms per crab at the Adelaide Bay, Flinders Island, site. Elsewhere, prevalences ranged from 0 to 20% with mean intensities up to 5 larvae per crab. Most larvae were cystacanths; often dead. A few acanthellae were detected.

The water rat, *Hydromys chrysogaster* (Thomas, 1909) acanthocephalan *Corynosoma stanleyi* (Smales, 1986) was recovered from this host only at Dunalley, where it was quite common (prevalence 59%, mean intensity 5.5 worms per crab). However this acanthocephalan may have been under-sampled. The larvae were often attached by connective tissue to the ovary, gut, body musculature, digestive gland and seminal receptacles of infected crabs, making them difficult to observe and extract by dissection. They were also more variable in size than were the other larval parasites in these crabs.

Paragrapsus gaimardii (Milne Edwards, 1837) (fam. Grapsidae): An entoniscid isopod not readily distinguished from the parasite of *C. granulosus* (*Portunion* sp. B), was widespread in Tasmania with prevalences ranging from 3% to 20%. Intensities were usually one female parasite per host. However, an infected crab at Dru Point, Margate, in south-eastern Tasmania, had four parasites, including two gravid adults. One of the infected crabs from Battery Point, Hobart, had a very recent infection: a

cryptoniscus larva being recovered from an anomalous location within the outer host-produced sheath that surrounded the cystacanth larva of the acanthocephalan *P. sphaerocephalus* walking on the surface of the acanthocephalan.

Spherical digenean trematode metacercariae, probably of *Gynaecotyla hickmani* (Smith, 1983), were present in 20 to 89% of *P. gaimardii* from most of the Tasmanian and Flinders Island sites. Most cysts were in the digestive gland but some were scattered throughout the haemocoel. Mean intensities were generally low – less than ten per infected crab. At Murdunna, south-eastern Tasmania, the metacercariae appear to be *M.eriolae*. Its prevalence was 40%, mean intensity 2.1. One *G. hickmani* cyst was found in one crab at Murdunna.

Metacestodes of *D. martini* were recovered from *P. gaimardii*. Prevalences ranged from 11% to 83%, with no obvious geographic pattern. Intensities were low, ranging from 1 to 6.4 per infected crab. The worms recovered from Adelaide Bay closely resembled the material from the portunid crabs. At Hanns Inlet, Western Port, Dunalley and Murdunna these larvae were smaller, and similar to the trypanorhynchs from *C. granulosus* at Tasmanian sites. At Barilla Bay and Dru Point, the trypanorhynchs referable to *D. martini* were very slender when excysted, being but a quarter the width of a host digestive gland tubule. The tissue between the muscular bulbs was dark red and the cyst wall was tough, resisting the probe at dissection.

One crab at Adelaide Bay (Flinders Island) and another at Dunalley (Tasmania) were each infected with a single encysted larva of *T. aetobatidis*.

Of the *P. gaimardii* at Murdunna, 6% had a single larva of an unidentified encysted trypanorhynch metacestode (*Trimacracanthus* sp. A). It was recovered from the pyloric region of the digestive gland. The hook armature is indistinguishable from *T. aetobatidis* (I. Beveridge, pers. comm.), but the worm differs from it in being very small (2–3 mm excysted extended length) and having a white blastocyst, relatively large bothridia and clusters of brown cells on the surface of the anterior third of the muscular bulbs.

Tetraphyllidean tapeworm larvae were found in 33% (mean intensity 5.5) of the *P. gaimardii* from Barilla Bay. All were located in the proximal part of the digestive diverticulae. These larvae had four bothridia and a small apical sucker. They lacked hooks. Since the ends of the paired digestive diverticulae are sometimes hard to find, these worms have probably been undersampled.

The acanthocephalan *P. sphaerocephalus* was commonly found in 11 to 50% of *P. gaimardii* at Orford, south-eastern Tasmania, and Hanns Inlet, Western Port. Mean intensities were generally low (1–3.9 per infected crab). Most were in the cystacanth stage; only a few acanthellae were recovered. Dead parasites were common.

A single crab infected with a single cystacanth of *C. stanleyi* was found at each of Adelaide Bay and Dunalley.

Gregarine trophozoites were commonly observed in the digestive diverticulae of *P. gaimardii*. Their abundance was not quantified. As with the tetraphyllidean larvae, these were undersampled.

Paragrapsus quadridentatus (Milne Edwards, 1837) (fam. Grapsidae): This crab was sampled at three sites in Tasmania. No entoniscids were recovered. Digenean metacercariae were prevalent at Dunalley, and at Perkins Point in north-western Tasmania, although mean intensities were always low (below three per infected crab). All metacercariae were taken from the digestive gland. They were similar in appearance to *Maritrema eriolae* (Smith, 1983).

A single *D. martini* was recovered from a crab at Perkins Point, and another worm referable to *D. martini* on the basis of hook morphology was collected from a crab at Dunalley. That worm resembled *D. martini* in *P. gaimardii* from the same site.

Trimacracanthus aetobatidis was also recovered from two *P. quadridentatus* at Dunalley. Mean intensity was two and most of the worms were in the subcardiac region of the digestive gland.

Single specimens of *P. sphaerocephalus* were collected from one crab each at Battery Pt. and Dunalley. *C. stanleyi* was found in two crabs from Dunalley.

Paragrapsus laevis (Dana, 1851) (fam. Grapsidae): *Paragrapsus laevis* was only sampled on the Victorian coast. No entoniscids were found in these small collections.

At Queenscliff, one crab was infected with three unidentified, spherical, pigmented metacercariae in the squashes of ovarian tissue.

D. martini was found in 67% of the crabs from Queenscliff, with a mean intensity of 6.5 worms per infected host.

At Hanns Inlet, Western Port, *B. laevis* was heavily infected with *P. sphaerocephalus* (prevalence 89%, mean intensity 9.5 worms per infected host). One crab had a remarkable 37 cystacanths. All the cystacanths were dead in another crab. A few acanthellae were also recovered from this site.

Leptograpsus variegatus (Fabricius, 1793) (fam. Grapsidae): These crabs were not commonly encountered. A few specimens from Adelaide Bay, Flinders Island, and a single specimen from Dru Point, Tasmania were examined for parasites. Of the Adelaide Bay crabs 60% were infected with *D. martini* (mean intensity 1.7). The single specimen from Dru Point was uninfected.

Brachynotus spinosus (Milne Edwards, 1853) (fam. Grapsidae): An undescribed entoniscid (cf. *Portunion* sp. C) was found in a *B. spinosus* at Murdunna. It was an adult female, with a mature brood of epicaridean larvae. It is readily distinguishable from *Portunion* sp. B found in *P gaimardii* and *C. granulosus* by the marked lateral prolongation of the ascendant lobe of the first oostegite well beyond the tip of the abdomen, at the point where it attaches by the chitinous calyx to the gill chamber of the host. Like *Portunion* sp. B, its ovary is lemon yellow.

Metacestodes of *D. martini* were present at Murdunna and Dunalley Channel, south-east Tasmania, and Adelaide Bay, Flinders Island, their prevalences ranged from 12% to 60 %. The highest prevalence was at Adelaide Bay. Mean intensities were low (1–1.3 cysts per infected crab); most crabs harboured a single worm. At Dunalley the trypanorhynchs assignable to *D. martini* on the basis of their proboscis armature were all of the very small variety noted in the other grapsids from Dunalley and some other south-eastern Tasmanian sites.

P. sphaerocephalus was found at Adelaide Bay and Dunalley; prevalences ranged from 15% to 40%. Mean intensities ranged from 1.5 to 3.0 per infected crab; rather high levels for such small hosts.

Helograpsus haswellianus (Whitelegge, 1889) (fam. Grapsidae): *Helograpsus haswellianus* was parasitised by an undescribed species of entoniscid isopod (cf. *Portunion* sp. D) at all sites investigated. Prevalences of the female isopods ranged from 11% to 33% and only single infections were detected. The posterior lobe of the ovary is relatively much longer than in the other species of *Portunion* collected during this survey. The ascendant oostegite is also diagnostic. It is greatly elongated as in *Portunion* sp. C, but while in the latter it extends anteriorly and then laterally to the cephalon, in *Portunion* sp. D the ascendant lobe of the oostegite extends anteriorly, laterally and then reflects posteriorly to such a great extent that its tip reaches the level of the anterior lobe of the ovary in the thorax. The ovary of these parasites was lemon yellow and the digestive gland was a deeper shade of orange than seen in other *Portunion* spp. All infected female crabs lacked any sign of ovarian development.

At Patriarchs Inlet (Flinders Island) 6 and 18 % of the crabs were infected by the metacercariae *G. hickmani* and *M. erioliae* respectively. There were 6 or fewer per infected crab.

Smooth Pebble crab, *Bellidilia laevis* (Bell, 1855) (fam. Leucosiidae): This crab was abundant in sandy flats in southeastern Tasmania and was also trapped in shallow water at Western Port.

A previously unknown rhizocephalan barnacle was recovered from Chilcott Rocks (Western Port) Adelaide Bay (Flinders Island) and Barilla Bay (south-eastern Tasmania) with prevalences ranging from 9% to 16%. No parasitised males were found. Almost all were single infections; one crab at Barilla Bay had two externas. Another crab, with a single externa, was held in the laboratory and produced a second externa. Virgin externas are a pale greenish-yellow. After the externa is fertilised, it becomes yellow, and as the eggs inside the externa develop, it changes to light pink. As naupliar release approaches, it becomes light purple with black spots (the naupliar eyespots). The interna was a delicate cobweb of roots that was difficult to distinguish from the digestive gland tubules of the host.

With fully developed externas, the crab ovary was degenerate, often being difficult to locate. The seminal receptacles of all infected female crabs were full of sperm. Of the 32 parasitised female crabs from the Barilla Bay sample, 25% were post-ovigerous (indicated by empty crab egg shells attached to the pleopods). All infected female crabs had a morphometrically normal abdomen with locking pits, normal genital

openings and their pleopods were not modified. No postmoult crabs had an externa. All crabs harbouring rhizocephalans, even with an interna or a virgin externa, were in the C_4 moult stage.

The unnamed species of *Carcinonemertes* described by Bell and Hickman (1985) was collected from Barilla Bay and Pipe Clay Lagoon (both southeastern Tasmania) at similarly low prevalences (4%–6%, 8% on non-ovigerous females, n =134) and mean intensities (1.3–4.0). All were juveniles or regressed adults. They were only found under the abdomen of female crabs. They were comiform, 1.0–1.5mm, enclosed, in a tough curved sheath, head end (with eyespots) towards the host cuticle, being attached by a pedicle to either the abdominal or thoracic sternites. The pedicle was highly elastic: it stretched over 10 times its original length before snapping. They were inactive in the sheath and remained sluggish even upon extraction (unlike most carcinonemerteans). Two double infections of carcinonemerteans with rhizocephalans were found. In both cases the host was post-ovigerous, retaining egg membranes attached by their funiculi to the pleopods. Additional information on this worm (as *Carcinonemertes* sp B) is given in Sadeghian and Kuris (2001). They observed the adult worms on ovigerous females and noted their unusual corkscrew shaped sheath.

Trematode metacercariae were recovered from *B. laevis* at most sites (excluding Chilcott Rocks where the crabs were not examined for metacercariae). The 34 crabs from Barilla Bay, Tasmania, were examined for larval flukes and tapeworms. Prevalences ranged from 18 to 56% and intensities were generally low: from 1.3 to 9.5 per infected crab. At all 3 sites (Barilla Bay, Pipe Clay Lagoon and Adelaide Bay)

cysts resembling both *G. hickmani* and *M. eroliae* were present in the digestive glands. One crab from Pipe Clay Lagoon had 24 small oval cysts, which may be the small oval cyst species Smith (1981) found in the carapace region of the digestive gland. Although not quantified, the thoracic ganglion and attached nerves of most *B. laevis* from Pipe Clay Lagoon were heavily infected with *M. paragrapsi*. One *M. paragrapsi* cyst was also seen in the nerve of a crab from Barilla Bay.

Dollfusiella martini (Beveridge, 1990) were only recovered from Adelaide Bay (Flinders Island) where the prevalence was 28% and mean intensity 1.8 per infected crab.

Populations of an unidentified gregarine were seen in the proximal portions of all the digestive gland diverticulae examined in *B. laevis* from all sites.

Giant crab, *Pseudocarcinus gigas* (Lamarck, 1818) (fam. Xanthidae): Eight ovigerous female crabs collected from sites off the eastern coast of Tasmania by the Tasmanian State Fisheries Laboratory at Taroona had adult carcinonemertean worms in all egg masses. The egg masses were in mid to late stages of embryonic development. The adult female worms were 7–30 mm long and pink while the males were smaller (6–10 mm) and pinkish-white. Two small eyespots not easily visible, were present. Adult females were ensheathed. The sheaths had prominent oval, evenly sized, lapillae on the external surface. The sheaths were not aligned along the fascicles of egg-bearing setae, but were intertwined among the crab eggs. The sheath is tough, elastic and sticky, with host eggs adhering to its surface. The gonads were not readily

visible and not in the typical ladder-like arrangement of carcinonemerteans. Behind the head the body widened, forming a shoulder, even in the males. The posterior end of the female worms was typically pointed; the posterior end of the males was blunt. The seminal vesicle was evident, although Takakura's duct was not readily apparent. The worms were robust and not readily squashed by coverslip pressure and were not sticky. When removed from their sheaths, female worms were more active than were the males. Female worms coiled and contracted strongly in fixative. A stylet, wide at the base and concave posteriorly, was present.

Long nemertean egg strings were present among the host eggs, often aligned with the setal fascicles, running up and doubling back along the 10–20 mm long fascicles. The worm larvae were of the simple ciliated carcinonemertean larval type, with long anterior and posterior cilia. When the larvae were at rest, the anterior cilium was half as long as the larval wórm. Eyespots were not evident in the larvae.

Four fascicles from a crab with advanced embryonic development (yolk reduced to two connected lobes) were examined to quantify brood losses due to the carcinonemerteans. A mean of 18% of the egg cases were empty; they had presumably been eaten by the worms.

Two frozen adult non-ovigerous female crabs were dissected. Both had ripe ovaries, clearly about to oviposit their clutches. No worms were found in a careful examination of limb axillae, pleopod setae and the surface of the sternal-abdominal furrow. Numerous encysted larval worms were found in the gills. The worms are

tightly coiled in circular cysts attached to the gill lamellae. Lying in the cysts, the worms take the shape of a tightly coiled comma. Eyespots are visible and the adhesive substance extends beyond the margin of the cyst. The cysts which are light pink, were most numerous on the larger lamellae near the ventral margin and near the rachis of the branchiae.

Amphipods were numerous in the egg masses of the ovigerous female crabs.

Other Crabs: Less than 6 individuals of each of the following species were examined (Table 1): *Halicarcinus* sp. (fam. Hymenosomatidae), *Naxia aurita* (Latreille, 1825) (fam. Majidae), *Notomithrax ursus* (Herbst, 1788) (fam. Majidae), *Mictyris platycheles* (Milne Edwards, 1852) (fam. Myctyridae), *Pilumnopeus serratifrons* (Kinahan, 1856) (fam. Xanthidae). No parasites were detected from any of these crabs.

DISCUSSION

Green crab parasites

The introduced green crab *C. maenas* is less parasitised than the related native crab *N. integrifrons* with which it shared some habitat wherever these two crabs were sampled. Neither of the two parasitic castrators of the native crab (an undescribed entoniscid isopod and a rhizocephalan, see chapter 5) was found in *C. maenas*, although the genus *N. integrifrons* is the most closely related to *Carcinus* (cf. Stephenson and Campbell, 1960).

In Port Phillip Bay, the green crab was infected by the larval tapeworms and nematodes whose final host were elasmobranchs. On the east coast of Tasmania, where the several grapsid crabs were often parasitised by larval digenean trematodes and acanthocephalans whose final hosts are birds or mammals, no green crab examined had acquired any of these abundant parasites.

The absence of bird parasites from the green crab populations in Australia is surprising because these parasites are widely reported in green crab populations in Europe (Crothers 1967, Lim and Pike 1980). Further, microphallid metacercariae and a species of *Polymorphus* are the only parasites that have transferred from native crabs to the green crab in New England (Pichelin *et al.*, 1998, Torchin *et al.*, 2002).

Our results indicate that native parasites infect introduced crabs with difficulty or not at all (whereas their infection rates in native hosts is high). This conclusion is supported by the studies of the green crab from all regions where it has been

introduced, including its sibling species *C. aestuarii* in Japan, and of *Hemigrapsus sanguineus* (De Haan, 1853) (native to Japan, introduced in northeastern USA) (Torchin *et al.*, 1996, 2001, 2003, Kuris and Lafferty unpubl. obs.). It appears that a significant advantage for exotic marine species is that they have been released from their natural enemies, most notably parasites, while their potential native competitors are afflicted with high parasite loads. In addition marine species introduced to a new environment may also experience less predation than in their native habitat (Torchin *et al.*, 1996) which could lessen the selective pressure to serve as intermediate hosts for trophically transmitted parasites.

The evidence suggests taxonomic relatedness is more important than ecological overlap for parasites to transfer to the green crab. The green crab in Victoria and Tasmania is most frequently collected with the native crab *P. gaimardii.* Yet it shares only those *P. gaimardii* parasites that are more abundant in *N. integrifrons* than they are in *P. gaimardii.* The green crabs from Victoria have acquired more local parasites than have green crabs from any other regions of the world where they were introduced (Torchin *et al.*, 1996). This is probably due to the close taxonomic relationship of the green crab with the native confamilial species *N. integrifrons*. The subfamily Carcininae has a Tethyan distribution and, for example, there are no carcinine representatives were *C. maenas* has been introduced on the Pacific coast of North America. The availability of taxonomic relatives for a pool of parasites available for transfer appears to be a principal condition for likely transfer of native parasites to exotic hosts.

N. tuberculosus and *O. australiensis*, which are deeper water, open-coast species, were completely free of parasites. They appear to play little role in either bird or elasmobranch parasite life cycles and their ecology is such that they are at little risk of infection by the many parasites of the portunids, grapsids and leucosiids of sheltered waters.

Host and Geographic Patterns

The parasite fauna of these inshore crabs can be grouped into three trophic adaptive syndromes: parasitic castrators, symbiotic egg-predators and, trophically transmitted parasites. The latter clustered among hosts and sites based on whether their predatory final hosts were elasmobranchs or birds and mammals.

The parasitic castrators and egg-predator nemerteans all appear to be relatively host specific. The rhizocephalans, two nemerteans and three of the four entoniscids occurred in only one host species. The remaining entoniscid (*Portunion* sp.) parasitised *C. granulosus* and *P. gaimardi*, both of which are in the subfamily Sesarminae. A congener, *Portunion conformis* (Muscatine, 1956), has a similar host specific pattern on the Pacific coast of North America: it is common in *Hemigrapsus oregonensis* (Dana, 1851) and occurs in *H. nudus* (Dana, 1851) but has never been found in *Pachygrapsus crassipes* (Randall, 1840), although this crab has broad habitat overlap with those *Hemigrapsus* species (Kuris *et al.,* 1980).

The geography of *Sacculina* sp. from *N. integrifrons* is interesting. We have recovered it only from the North Arm of Western Port, where it was abundant. This may account

for the paucity of Australian records for this large, distinctive parasite with a highly visible externa. Haswell, (1888) recorded parasitised individuals from Port Jackson, immediately referring his observations on the feminised males to Giard's (1886) recently formulated concept of parasitic castration. Other than a possible photograph of a sacculinised *N. integrifrons* in Hale (1927), there appears to be no other record of this parasite. Although Hale examined many *N. integrifrons* from the South Arm of Western Port, G. Edgar (pers. comm.) he never reported a *Sacculina* specimen. Similarly, the extensive survey of Griffin and Yaldwyn (1971) of crabs in Port Phillip Bay does not record *Sacculina* on *N. integrifrons.* Thus, its geographic distribution appears to be extremely patchy, with high prevalences at very few sites.

In contrast to the localised distribution of *Sacculina* on *N. integrifrons*, the entoniscids from all host species appear to be rather widespread, as is the rhizocephalan parasite of *B. laevis*.

The trophically transmitted elasmobranch parasites include at least four species of larval trypanorhynch tapeworms, a larval tetraphyllid tapeworm, and larval nematodes in the genus *Proleptus*. Samples of crabs from Flinders Island and the Victorian coast are dominated by these parasites. The carcininine portunids *N. integrifrons* and *C. maenas* are the primary hosts, but the two most abundant species are also commonly seen in the grapsids, particularly *P. gaimardii*. In Tasmania, by contrast, the trypanorhynch tapeworms are less commonly found in *N. integrifrons* and *C. maenas* harbours only *T. aetobatidis*. The grapsids are the main hosts of *D. martini* and to a lesser extent *T. aetobatidis*.

The bird and mammal parasites (at least four species of digenean trematode metacercariae and the two acanthocephalans) largely use the grapsids as their hosts. Not one of these parasites occurred in *C. maenas* and only a single acanthocephalan cystacanth was recorded from *N. integrifrons*. These parasites occurred at most locations, suggesting that the predatory impact of birds and mammals on the native crabs is widespread. Heavy burdens of bird acanthocephalan parasites and trematode metacercariae were noted at Adelaide Bay on the southern coast of Flinders Island and the western end of the Denison Canal and adjacent shore of Blackman Bay at Dunalley. *Paragrapsus gaimardii* and perhaps *B. spinosus* appeared to be the most frequently parasitised hosts, and *C. granulosus* the least parasitised by the bird parasites, although some of the grapsids need more sampling to substantiate the comparison. The common use of the same prey intermediate hosts by parasites destined to occupy very different final hosts (e.g., birds and elasmobranchs) suggests that the concepts of hitch-hiking, co-piloting or hijacking through behaviour modification (Lafferty *et al.*, 1995, Thomas *et al.*, 2002, Brown *et al.*, 2005) may occur in these hosts.

Parasitic Castration

The new species of Rhizocephala from the leucosiid *Bellidilia laevis* presents some highly unusual life-history features. It appears to infect only female crabs. It is possible that feminisation is so complete that all infected males have an abdomen that bears female genital openings, pleopods, abdominal width and segmental joints and lack any evidence of male genital openings and gonopods. This seems unlikely because feminisation of the secondary sexual characteristics of male hosts in other rhizocephalan systems is usually variable (Høeg, 1995). Perhaps the precisely locking

abdominal flap of female *B. laevis* is so well suited to protect the externa that there has been selection to avoid males with their very narrow abdomens.

This unusual infection pattern may be a reflection of an unusual sex ratio whereby females predominate and are therefore more likely to be infected. At the two Tasmanian sites, females out numbered males by 3 to 1, possibly explaining the female infection pattern. However, at the Victorian site, 67 crabs were collected only 10 of which were female. None of male crabs collected from this heavily male biased population were infected suggesting that sex ratios may not explain the female infection pattern. It is possible that infected males may have migrated to deeper waters and therefore did not appear in our sampled populations. Without an understanding of the general population structure and dynamics, it is difficult to confidently explain the infection pattern we report.

Parasitic castration has been confirmed in all well-studied rhizocephalan host-parasite associations. The lack of detectable gonads in *B. laevis* parasitised by rhizocephalans indicates that such is the case with this association. However, there are two unusual features of the effect of the rhizocephalan on the host's reproduction. Firstly, the sperm-filled seminal receptacles of all the infected females suggest that these females had either mated before becoming infected or could mate successfully after becoming infected. Secondly, and quite perplexing, was the large number of female crabs with externas that retained some empty crab eggshell membranes attached to the pleopods. This means that in the instar, in which the rhizocephalan externa emerged, there had been at least a partial brood of crab embryos oviposited and successfully reared.

When sterile, unfertilised eggs are oviposited, they are soon sloughed; when embryos are aborted during embryogenesis, the integrity of the egg shell is retained even after the viable embryos have hatched.

If *B. laevis* is producing a viable egg mass before the emergence of the rhizocephalan externa, the physiological relationship between host and parasite is quite different from other described parasitic castration systems where the reproductive physiology of the host is changed quite soon after the parasite has become established in the host (Kuris 1993, Høeg 1995). Based on the consistent sizes, reproductive rates and lack of juvenile crabs in our samples, it seems that *B. laevis* has a strongly seasonal life history. If the timing of rhizocephalan infections was precise, its development very rapid, and the most susceptible instar was the pre-pubertal stage, it is possible that the host might often be able to produce one clutch of eggs (or at least a partial clutch) before the full parasitic castrator effects took hold. The observations of the continued degeneration of the crab ovary from crabs with internas only to crabs with mature externas supports this hypothesis of rapid parasite development being 'coordinated' with late cessation of host reproduction. In any case, this rhizocephalan may have a more limited effect on the host's reproductive output than do other rhizocephalans.

All virgin externas were recorded from crabs in the C_4 moult stage. Thus, *B. laevis* can be added to the short list of species that emerge from hard crabs (Day 1935, Veillet 1945, Høeg and Lützen 1985). However, none of these studies determined the actual moult stage, so though characterised as hard, these crabs may well have been in late postmoult stages (C_1–C_3) rather than the actual intermoult, C_4, stage.

Biological Control

The parasites found in this survey that are most likely to be exerting any control over crab populations are those infecting crabs as their primary hosts: nemertean worms, entoniscid isopods and rhizocephalan barnacles. The egg-preying nemerteans and the parasitic castrating isopods and barnacles would, to varying degrees, limit the reproductive capacity of infected individuals and possibly populations (Kuris and Lafferty 1992). However, none of these parasites were found in introduced crabs considered pests in Australia.

Of the two introduced crab species, *C. maenas* and *M. novaezealandiae,* only *C. maenas* was parasitised. The parasites found in *C. maenas* - larval trypanorhynch tapeworms and nematodes – are encysted and do not affect crab reproduction. Heavy trypanorhynch infections cause some pathology of the digestive gland. This pathology may affect crab health at both the individual and population level thereby potentially exerting some control over the introduced pest, *C. maenas*.

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Appendix A.

Site	Latitude & Longitude	Collection Method	Habitat Notes
Tasmania			
Ansons Bay	41°02′S, 148°16′E	hand	Sandy bottom, sea grasses
Barilla Bay	42°49′S, 147°28′E	hand	Oyster lease, sand over clay
Battery Point	42°53′S, 147°20′E	hand	Rocky beach, small to medium* sized rocks
Bicheno	41°52′S, 148°18′E	hand	Abalone hatchery outflow over granite rock foreshore
Blackman Bay	42°52′S, 147°51′E	trap	Rocks over sand, light seaweed cover
Coast 1 km north of Old Man Creek	42°14′S, 148°.00′E	hand	Rocky beach
Dru Point	43°01′S, 147°16′E	hand	Small rocks over sand with light seaweed cover
Dunally	42°54′S, 147°48′E	trap	Small basaltic rocks over sand and gravel
Great Oyster Bay	42°11′S, 148°09′E	trawl	
Henderson Lagoon	41°29′S, 148°15′E	Hand	Site 1. sandy bottom with decaying kelp
		& trap	Site 2. sedge grasses and muddy soil
Humbug Point	43°31′S, 147°85′E	trap	Sandy bottom, thick seaweed cover, 2–3 m depth
Little Swanport	42°19′S, 147°58′E	trap	Oyster lease, sandy bottom , 1–2 m depth
Murdunna	42°94′S, 147°85′E	hand	Small basaltic rocks over sand, sedge grasses at beach e
Orford	42°33′S, 147°52′E	trap	Sandy bottom, scattered rocks
Selfs Point	42°51′S, 147°19′E	trap	Muddy bottom 2–3 m depth

Perkins Point	40°45′S, 145°17′E	hand	
Pipeclay Lagoon	42°98′S, 147°52′E	hand	Sandy enclosed bay
Flinders Island			
Adelaide Bay	40°14′S, 148°14′E	hand	Small to medium basaltic rocks over sand
Cameron Inlet	40°06′S, 148°16′E	Trap	Sandy bottom with freshwater drainage in
Port Phillip Bay, Victoria			
Kirk Point	38°02′S, 144°33′E	hand	Breakwater rocks
Swan Bay	38°13′S, 144°39′E	hand	Sandy bottom
Williamstown	37°51′S, 144°53′E	Hand	Site 1. jetty, rocks over sandy bottom
		& trap	Site 2. beach small to medium* sized rock
Western Port, Victoria			
Chilcott Rocks	38° 21′S, 145°16′E	trap	Sandy bottom, scattered rocks, light seawe
Hanns Inlet	38°22′S, 145°11′E	trap	Small to medium-sized rocks over muddy
Hastings	38°18′S, 145°11′ E	trap	Sandy bottom, 3–5 m depth
Merricks Creek	38°22′S, 145°08′E	trap	Sandy bottom, 100 m from shore, 3–5 m d
Sandstone Island	38°19′S, 154°12′E	trap	Sandy bottom, 3–5 m depth
Sandy Point	38°24′S 145°14′E	trap	Sandy bottom, steep bank, 3–5 m depth
Somers	38°.23′S 145°09′E	trap	Sandy bottom, 100 m from shore, 3–5 m d
Stony Point	38°28′S 145°24′E	trap	Sandy bottom, 0–5 m depth
Tortoise Head	38°24′S 145°16′E	trap	Sandy bottom, scattered rocks, light seawe

CHAPTER 3

THE HISTOPATHOLOGICAL EFFECTS OF TRYPANORHYNCH METACESTODES IN THE DIGESTIVE GLAND OF A NOVEL HOST *CARCINUS MAEANS* (DECAPODA: PORTUNIDAE)

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THE HISTOPATHOLOGICAL EFFECTS OF TRYPANORHYNCH METACESTODES (CESTODA: TRYPANORHYNCHA) IN THE DIGESTIVE GLAND OF A NOVEL HOST *CARCINUS MAENAS* (DECAPODA: PORTUNIDAE)

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ABSTRACT: The green crab *Carcinus maenas* (Linnaeus, 1758) was introduced to Australian temperate waters in the late 1800's, has since established and is now considered to be a pest. We undertook an extensive parasite survey to find potential natural enemies of *C. maenas* and found it is infected in Australia by two species of larval trypanorhynch tapeworm: *Trimacracanthus aetobatidis* (Robinson, 1959) and *Dollfusiella martini* (Beveridge, 1990). We describe the gross pathology and histopathology of the parasites' new host (*C. maenas*) and note that the plerocercoid larvae are located in the lumen of the digestive gland tubules. The presence of *D. martini* in *C. maenas* with low population numbers suggests that either *D. martini* has an impact (direct or indirect) on the survival of *C. maenas*, or that the parasite may have been an indicator of high predation pressure. If the former were true, this would contribute to the control of this introduced pest species.

Key Words: Trypanorhynch, *Carcinus maenas*, biological control, crustacean pathology

INTRODUCTION

The green crab *Carcinus maenas* (Linnaeus, 1758), a native of the Atlantic coast of Europe, has been introduced to North America, South Africa and Australia, where it is recognised as an invasive pest (Tettlebach 1986). A related Mediterranean species, *C. aestuarii* (Nardo, 1847), has become established in Japan (Geller *et al.,* 1997). Both *Carcinus* species are generalist predators, with an appetite for bivalves, gastropods and other invertebrate infauna. Apart from directly threatening prey species, *C. maenas* has the potential to change the structure of native communities (Le Roux et al. 1990, Grosholz and Ruiz 1995, MacKinnon 1997, Walton 1997) and may even affect the abundance, demography and behaviour of migratory shorebirds (Ruiz 1987).

Species which have been introduced to new environments are often more successful than they were in their native environments (provided the new environment is suitable for growth and reproduction), in part, because they have escaped the predators, parasites and diseases of their endemic range (Torchin *et al.,* 2001, Torchin *et al.,* 2003). Support for this hypothesis was provided by Kuris and Gurney (in press), who surveyed green crabs, *C. maenas* and native crabs in Australian temperate waters and found green crabs to be relatively free of parasites compared to related *Nectocarcinus integrifrons* (Latreille, 1825), and ecologically similar (Grapsidae) crabs.

However, at one site (Swan Bay, Victoria), green crabs were heavily parasitised by larval tapeworms and at this site (unlike other areas in Australia) the green crabs were less abundant compared to other crabs. Here numbers were so low that the standard sampling

protocol (baited traps left overnight) had to be abandoned and the area searched exhaustively to catch a small number of green crabs. Native crab numbers at this site were also relatively low but were nonetheless well represented by five crab families. A possible reason for the low abundance of green crabs at this site may be due to the pathology of the digestive gland caused by trypanorhnych tapeworm metacestodes. If this hypothesis is correct, then the tapeworms might aid control of green crab populations in temperate Australian waters.

Biological control of *C. maenas* using trypanorhynch tapeworms will be difficult due, in part, to the trophic transmission of the parasite which involves a number of hosts. The life cycle of both *Dollfusiella martini* (Beveridge, 1990) and *Trimacracanthus aetobatidis* (Robinson, 1959) is still to be elucidated, however, if these species of trypanorhynch develop through a free swimming coracidia stage, they may well follow a lifecycle similar to that of the trypanorhynch *Lacistorhynchus tenuis,* reported by Sakanari and Moser (1989). This would involve the coracidia of *D. martini* and *T. aetobatidis* infecting a copepod as the first intermediate host. The copepod would then be consumed by a crab, in this case *C. maenas*, where the procercoids develop into plerocercoids, and *C. maenas* becomes the second intermediate host. *C. maenas* is in turn eaten by shallow water rays and sharks where the plerocercoids excyst and develop into adult tapeworms living in the spiral valves of the final host.

Parasites with complex lifecycles involving more than one host present a number of problems for biological control. The most obvious problem is that if an intermediate host

is removed from the chain of trophic transmission, the final host will not be attacked or infected. Another problem relates to ensuring the specificity of the parasite in relation to a number of hosts instead of a single final host. Parasitoids, for example, have often proved to be successful biological control agents, in part, because they have a simple and direct lifecycle involving a single specific host.

Few reports of the pathology produced by trypanorhynchs in their final and intermediate hosts are available. Adult trypanorhynch tapeworms only occur in the spiral valves of elasmobranchs (Roberts and Janovy, 1996). Their range of intermediate hosts, however, is much greater: it includes bony fishes, molluscs and crustaceans (Cake 1976, Palm 1997). In this paper we describe the histopathological effects of the larval stages of trypanorhynch cestodes *D. martini* and *T. aetobatidis* (Beveridge and Campbell, 1987, Beveridge, 1990) on the digestive gland of *C. maenas* from Swan Bay, Victoria.

MATERIALS AND METHODS

In a preliminary survey, 43 adult *C. maenas* were collected from Swan Bay within Port Phillip Bay, Victoria (144° 39' 47", 38° 13' 8") in November 1996 (Fig. 1). They were collected by hand from underneath rocks at low tide and were taken live to the laboratory. The crabs were dissected by detaching the carapace to expose the body cavity, and the number of trypanorhynchs counted. On 16 July, 1999, 15 *C. maenas* from the same site

were collected and part of the digestive gland was removed and placed in seawater Davidson's fixative (Bell and Lightner 1988). The tissue was transferred to 70% ethanol after seven days and later processed for histology and sectioned at 6 μm. Sections were stained with hematoxylin and eosin (H & E), Masson's trichrome (Bradbury and Gordon 1991), periodic acid-Schiff reaction (PAS) (Cook 1991) and Von Kossa's reaction for calcareous corpuscles (Page *et al.,* 1991). In histological sections parasite density was scored as absent, light (1 worm per section), moderate (2 to 3 worms per section) or heavy (> 3 worms per section).

To identify the trypanorhynchs the entire larvae were placed in tap water to evert the proboscides and were then fixed in AFA (alcohol-formalin-acetic acid).

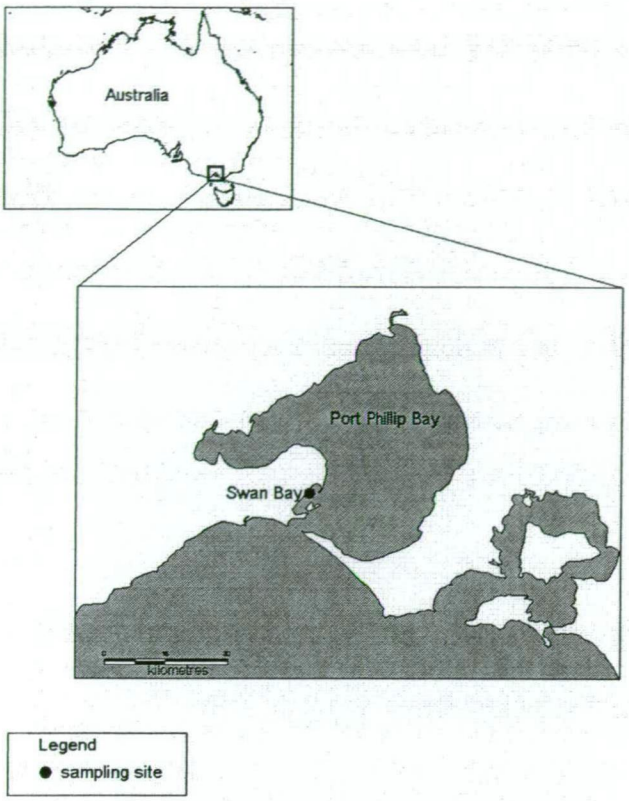


Figure 1. *Carcinus maenas* sampling site at Swan Bay, Victoria.

RESULTS

Two trypanorhynch species were found, *Dollfusiella martini* and *Trimacracanthus aetobatidis*. Their prevalences and intensities were 84% and 48%, and 8.8 and 2.2 respectively.

Gross Pathology

The digestive glands of lightly infected green crabs appeared normal, a glossy dark green colour, whereas the digestive gland of heavily infected crabs were a pale cream colour and speckled with black granules 0.5 to 1 mm long. Encapsulated worms were clearly visible within distended hepatopancreatic tubules. *Dollfusiella martini* appeared predominantly in the antero-dorsal segment of the digestive gland near the juncture of the cardiac stomach and midgut intestine, while *T. aetobatidis* plerocercoids were present mainly in the subcardiac sternal pocket of the thoracic sterna, anterior to the thoracic ganglion and ventral to the cardiac stomach.

Histology

The trypanorhynch species could be distinguished in sections. *Dollfusiella martini* was small (400 – 450 μm) and possessed large microtriches (Figs. 2 and 3). *Trimacracanthus aetobatidis* was larger (1100 - 1300 μm) and had a thick folded tegument without visible microtriches. (Figs. 2,3 and 4). The two species stimulated similar host responses. Most of the following description relates to *D. martini* as it was more prevalent: only well-developed plerocercoids were observed, and all were contained within the digestive

tubules. There was no evidence of migration into or out of host tissues. The surfaces of both species of worm were in direct contact with the tubule epithelium of the host's digestive gland; there was no sheath enveloping the worm nor separating it from the host (Fig. 6). Metaplasia of tubule epithelium was evident where the worms came into contact with the tubule. At these sites the normal cell population was gradually configured into a single layer of cuboidal and finally squamous cells which were so highly compressed that the nuclei become spindle-shaped. In addition to the tubular changes, which included atrophy and necrosis of epithelium, there was a loss of surrounding secretory parenchyma due to the pressure atrophy caused by dilated tubuli (Figs. 7 and 8). Longitudinal sections of tubules infected by both species of worm showed that distally or proximally to the worm, the tubule cells resumed their characteristic shape and resorptive (R) cells re-appeared (Fig. 6). This contrasted with uninfected tubules where the embryonic (E), blisterlike (B), fibrillar (F) and R cells, according to the schema of Jacobs (1928), were discernible in uninfected tubules, with B-cells being particularly abundant.

Pronounced inflammatory changes in the interstitium were observed in several cases where dense accumulations of haemocytes infiltrated the connective tissue surrounding the infected tubules (Figs. 9 and11). Granulomatous inflammatory reactions also occurred around tubules which had lost their integrity (Fig. 10) or where signs of degeneration, death or complete necrosis of plerocercoids occurred (Fig. 12). Granulomatous lesions surrounded by pigmented layers were observed in most infected specimens. Typical granulomas, with a necrotic centre and a thick layer of newly formed granulation tissue (Fig. 13) on the periphery, were also observed. These granulomas probably related to

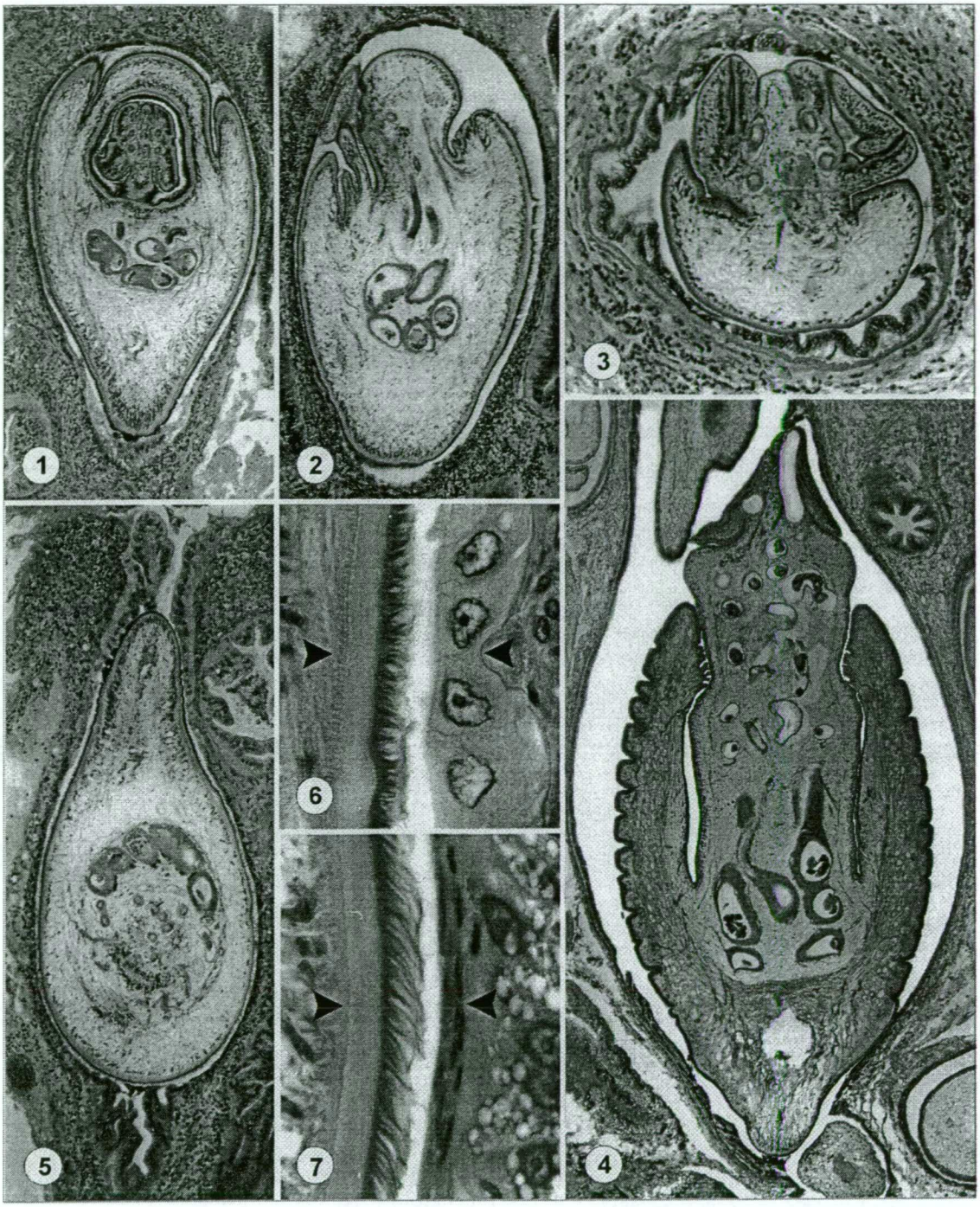
plerocercoid infection, but neither calcareous corpuscles, characteristic for the parenchyma of cestodes, nor calcareous hooks of the tentacles where observed in necrotic material.

Large numbers of haemocytes aggregated around infected tubules and, in particular, around melanised bodies (Figs. 12 and 13). Spherical eosinophilic cells, 35 to 40 μm in diameter, were associated with the haemocyte aggregations. The nucleii of these cells were positioned on the outer edge of the cell wall (Fig. 11). Light infections of either species of worm did not stimulate an inflammatory response. Individual worms occupied digestive tubules, but no aggregation of haemocytes surrounded the infected tubule.

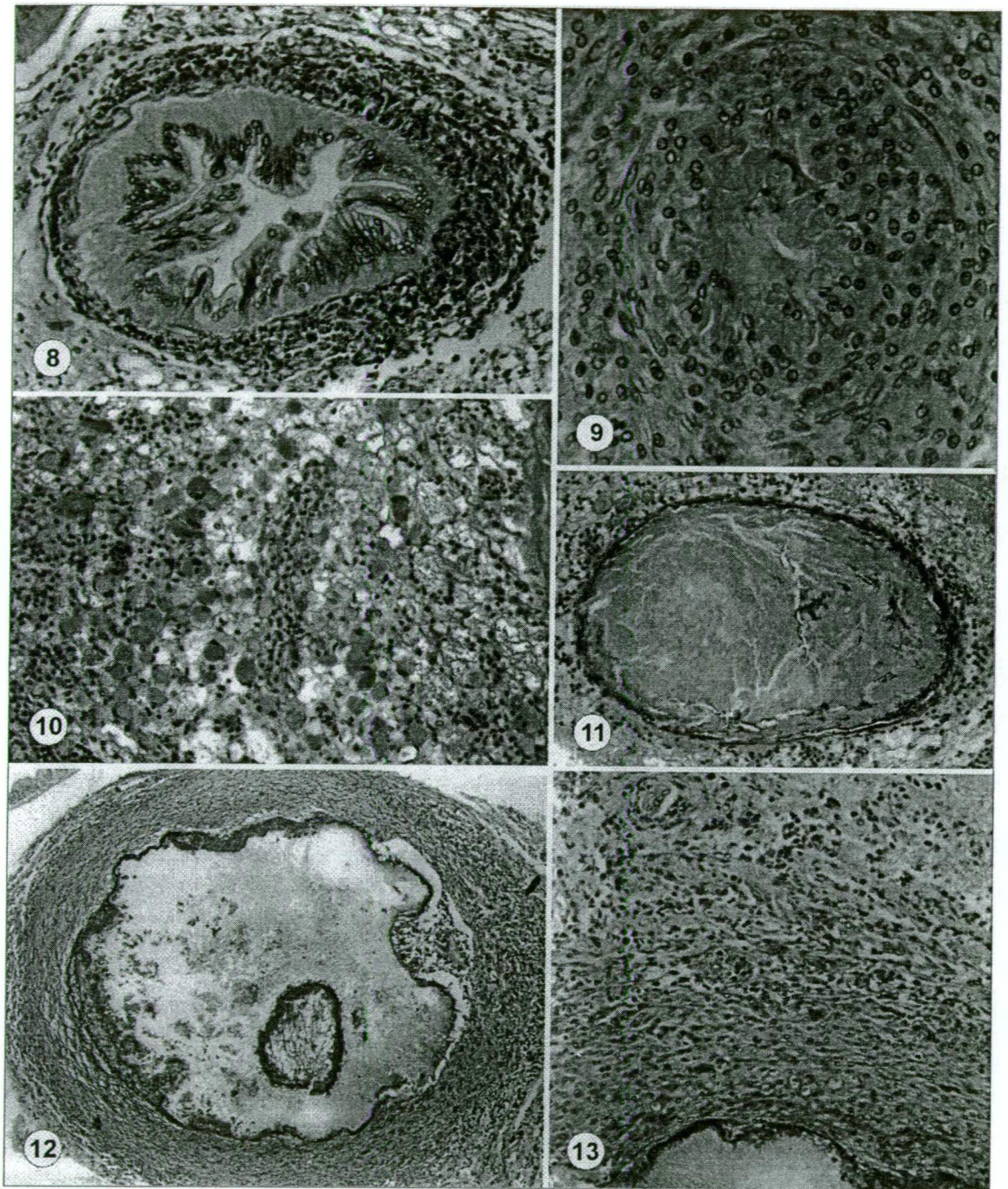
The black specks observed in gross pathology were too small to be intact metacestodes; they more closely resembled the shrivelled and melanised remains of digestive tubules with the hollow centre corresponding to the tubule lumen. Furthermore, the histological sections stained with von Kossa's reaction did not indicate the presence of either the hooks of the proboscides or calcareous corpuscles of worms in these melanised structures. These melanised specks were always in the same location and in direct contact with regions of the digestive gland containing heavy infections of *D. martini* plerocercoids, suggesting that they were the remnants of necrotic tubules damaged by cestode infection.

The digestive gland tissue of lightly infected and uninfected crabs showed very little staining with PAS; however, moderately and heavily infected crabs were darkly stained.

In heavily infected green crabs, PAS positive material was concentrated distally and proximally in R cells. Some congealed clumping of PAS-positive material also occurred in connective tissue between the tubules. Sectioned worms within the infected digestive gland were strongly PAS positive, as were the melanised bodies described above.



Figs. 2 to 8. *Dollfusiella martini* and *Trimacracanthus aetobatidis* infecting Carcinus maenas. Figs. 2 & 3. *D. martini* plerocercoids in the digestive glands of C. maenas. Plerocercoids are localised in distended tubules (H & E stain, X 35). Fig. 4. Cross section of *D. martini* anterior, showing bothridia and inverted scolex. Tubular epithelium partly atrophied (H & E, X 150). Fig. 5. *T. aetobatidis* plerocercoid in the digestive gland tubule of *C. maenas* (H & E, X 20). Fig. 6. Plerocercoid growth, which has produced pronounced hypobiotic changes of tubule epithelium around the most voluminous part of the plerocercoid while tubule epithelium remains normal in segments distal and proximal to the plerocercoid (H & E, X 35). Figs. 7 & 8. Host parasite interface. Left arrows mark tegument of plerocercoids with filamentous microtriches; right arrows mark flattened epithelial tissue of host digestive gland tubule (H & E, X 500).



Figs. 9 – 14. *D. martini* infecting *C. maenas*. Fig. 9. Inflammatory reaction surrounding a digestive gland tubule. The neighbouring segment is occupied by a plerocercoid (H & E, X 170). Fig. 10. Early granulomatous lesion surrounding a digestive gland tubule which has lost integrity (H & E, X 500). Fig. 11. Inflammatory infiltration of digestive gland tissue with predominating 'eosinophilic cells' (H & E, X 170). Fig. 12. A former digestive gland tubule containing necrotic material within a thin layer of connective tissue (H & E, X 35). Figs. 13 & 14. Granulomas surrounded by newly formed granulation tissue on the periphery (H & E, X 170; H & E, X 30).

DISCUSSION

Lesions developed in tissues adjacent to plerocercoids within the tubules. We think the pressure exerted by growing metacestodes upon the epithelial tissue of the infected tubules caused distention of the tubules, producing epithelial metaplasia and the loss of surrounding secretory parenchyma. The extent of lesions in the digestive gland tissue was directly related to the intensity of tubule infection. Light infections were not associated with histological changes, while heavy infections were clearly pathenogenic, supporting our gross observations of lightly infected digestive glands appearing normal in colour versus damaged heavily infected digestive glands being cream. The absence of immature plerocercoids suggests that the lightly infected crabs had been infected for some time without showing pathological signs or evidence of a strong immune response.

The green crabs' response to trypanorhynch infection in this study was typical of the Crustacea: it involved haemocyte aggregation, encapsulation and melanisation (Ratna and Vinson 1983, Soderhall and Cerenius 1992). The metacestode infection induced transformation of the tubule cells (recognisable E, F, R and B cells) into compressed squamous cells. At some point the plerocercoid died or was killed within the tubule, producing the necrotic highly PAS-positive material. Haemocytes aggregated around the infected tubule and produced a tight, multilayered capsule of fibrocytes, which in turn deposited melanin. Concentric encapsulation followed by melanisation occurred in the crayfish *Cherax quadricarinatus* (von Martens, 1968) hepatopancreatic tubules infected with intracellular rickettsiae (Owens *et al.,* 1992).

An endemic crab, *Nectocarcinus integrifrons,* is also parasitised by *T. aetobatidis* and *D. martini* (A.M. Kuris and R.H.Gurney, unpubl. data). However, little melanisation occurs in the digestive gland of heavily infected native crabs. Since *N. integrifrons* probably evolved in the presence of the trypanorhynch worms, *T. aetobatidis*, and *D. martini*, an attenuated inflammatory reaction might be an evolutionary response. This would appear to be adaptive as the stronger host response of the naive green crab presumably causes more disruption to digestive gland function than in the evolved host.

PAS staining suggested higher levels of glycogen in the digestive gland of crabs that were heavily infected with trypanorhynch metacestodes than in lightly or uninfected crabs. The grapsid crabs *Chasmagnathus granulata* infected with metacercariae was found to contain more glycogen than uninfected *C. granulata* (Robaldo *et al.,* 1999). The infiltration of infected tissue with glycogen-rich haemocytes may explain the increased glycogen levels in infected crabs (Robaldo *et al.,* 1999).

The digestive gland performs a range of important physiological functions and is a vital organ in Crustacea. It synthesises and secretes digestive enzymes, absorbs nutrients, is directly involved in excretion, moulting, storage of organic reserves and the metabolism of lipids and carbohydrates (Gibson and Barker 1979). These functions are likely to be severely impaired by heavy infections with trypanorhynch metacestodes. High intensity infections reduce the numbers of intact tubules, damage interstitial tissue and produce pronounced inflammation (as evidenced by increased haemocyte numbers). The higher levels of glycogen found in infected crabs also suggest abnormal carbohydrate

metabolism. Trypanorhynch infection may also impair the immune system, making the host susceptible to other infections.

Carcinus maenas found in Swan Bay, Victoria were more heavily infected with trypanorhynchs than *C. maenas* from other sites in Victoria, Flinders Island and Tasmania (A.M. Kuris and R.H.Gurney, unpubl. data). The abundance of *C. maenas* was lowest from from Swan Bay (Victoria) where intensity and prevalence of infection was highest. Though the low green crab abundance at Swan Bay may be due to a range of factors, the high prevalence of trypanorhynch infections may be a contributor, either directly by the pathology associated with this infection or by making hosts more susceptible to infection by other pathological agents, reducing their ability to withstand environmental stressors.

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CHAPTER 4

THE EFFECT OF PARASITISM BY TRYPANORHYNCH PLEROCERCOIDS (CESTODA: TRYPANORHYNCHA) ON THE DIGESTIVE ENZYME ACTIVITY OF *CARCINUS MAENAS* (DECAPODA: PORTUNIDAE)

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Authors: R.H. Gurney, D. J. Johnston, B.F. Nowak

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BY

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ABSTRACT: European green crabs, *Carcinus maenas* (Linnaeus, 1758), infected with trypanorhynch plerocercoids, *Dollfusiella martini* (Beveridge, 1990), were studied to determine whether larval cestodiasis had an effect on the digestive enzyme activity of the green crab host. We collected 28 green crabs from Swan Bay, Victoria, removed their digestive glands, counted the plerocercoids and assayed the digestive gland for three enzymes: trypsin, lipase and α-glucosidase. Regression analysis showed a significant negative relationship between levels of infectation and the specific activity of trypsin (r^2 = 0.17, d.f. = 1,22, $P < 0.05$, y = –3.91 E–06x + 1.683 E–04) and lipase (r^2 = 0.40, d.f. = 1,23, $P < 0.001$, y = –2.89 E–05x + 1.615 E –03), i.e., the higher the level of infectation, the lower the trypsin and lipase activity. No relationship was found for α-glucosidase activity. We also found a positive correlation (r^2 = 0.41, d.f. = 27, $P < 0.05$) between intensity of infectation and host size, a common host/parasite phenomenon. We conclude that infectation by plerocercoids of the trypanorhynch, *D. martini*, adversely affects digestive gland function of green crabs by reducing the specific activity of the digestive enzymes trypsin and lipase. This reduction in enzyme activity may be attributed to the destruction of enzyme producing F-cells resulting from plerocercoid infection of the digestive gland.

Key Words: *Carcinus maenas* (Decapoda: Portunidae), *Dollfusiella martini* (Cestoda: Trypanorhyncha), pathophysiology, hepatopancreas, trypanorhynch, digestive enzyme, trypsin, lipase, α-glucosidase.

INTRODUCTION

The cestode lifecycle typically involves one or more intermediate hosts and the effects of infectation on intermediate hosts can be severe, producing serious organ damage and extreme inflammatory responses. Neurocysticercosis is an example of the well-known clinical syndrome where growing Cyclophyllidean larval cysts (cysticerci) of *Taenia solium* (Linnaeus, 1758) localize in the central nervous system of humans with potentially fatal consequences (Sciutto *et al.*, 2000). This association with humankind stretches back into antiquity and continues to be a serious impediment to human health in both developing and affluent nations of the world (Sovillo *et al.*, 1992, Hoberg, 2002). It is not surprising that the Cyclophyllidea receive a great deal of scientific scrutiny.

By contrast, the effects of the cestode order Trypanorhyncha infecting the intermediate host (crustaceans, fish and molluscs) are largely descriptive and not extensively documented. The economic and social impact of this tapeworm is small in comparison with the Cyclophyllidea and is limited to devaluing fisheries products (Overstreet, 1978, Palm, 1997). Consequently, most reports provide intermediate host lists, taxonomic descriptions and geographic ranges (Tripp and Turner, 1983, Rigby and Dufour, 1996, Palm, 1997, Vidal-Martinez *et al.*, 2002). There are few reports describing the physiological and pathological effects of Trypanorhynch plerocercoids on their intermediate hosts.

The histopathological appearance of crustacean larval cestodiasis has been described for shrimp infected with trypanorhynch plerocercoids (Sparks and Fontaine, 1973) and

hermit crabs infected with tetraphyllidean plerocercoids (Smolowitz, *et al.*, 1993). In both cases the inflammatory reaction involved the migration of haemocytes accompanied by melanisation and encapsulation within the affected area. The same reaction has been observed in the digestive gland of green crabs, *Carcinus maenas* (Linnaeus, 1758), infected with trypanorhynch plerocercoids (Gurney *et al.*, 2004) and in this case, the pathology was evident at both the gross and histological levels.

The digestive gland performs a range of important physiological functions and is a vital organ for digestion in Crustacea. It synthesizes and secretes digestive enzymes, absorbs nutrients, is involved in excretion, storage of food reserves and the metabolism of lipids and carbohydrates (Johnston *et al.*, 1998). High intensity trypanorhynch and tetraphyllidean plerocercoid infectations of this organ have been shown to reduce the number of intact tubules, damage interstitial tissue and produce pronounced inflammation (Smolowitz, *et al.*, 1993, Gurney *et al.*, 2004). In this study we investigated whether there was a measurable physiological effect of impaired digestive gland function as a result of physical damage produced by cestodiasis. Specifically, we examined the relationship between digestive enzyme activity and the intensity of crab infectation as found in the green crab, *C. maenas*, infected with plerocercoids of the trypanorhynch *Dolfusiella martini*.

Adult *C. maenas* are omnivorous feeders, consuming infaunal invertebrates and small amounts of macroalgae (Grosholz and Ruiz, 1996, Mackinnon, 1997, Elner, 1981). Therefore we, analysed the specific activity of three digestive enzymes: lipase, trypsin

and α-glucosidase, in order to assess the metabolism of the three major food groups: lipids, proteins and carbohydrates, which constitute an omnivorous diet.

MATERIALS AND METHODS

Collection

Adult *C. maenas* were collected by hand at low tide from Swan Bay within the greater Port Phillip Bay in Victoria, Australia (144° 39' 47"E, 38° 13' 8"S) on October 16th 2002. The crabs were collected from underneath rocks scattered across the inter-tidal zone. Once all the crabs were caught from the inter-tidal zone (approximately one-hour catching effort, 8.00 a.m. to 9.00 a.m.) they were wrapped in aluminum foil and snap frozen at –298°C in liquid nitrogen. Twenty-nine crabs (male and female) were collected, ranging in size from 40 mm to 72 mm carapace width. Only hard shelled (presumed intermoult) crabs were collected. Soft-shelled (post-moult) crabs or ovigerous crabs were returned to the collection site.

Prevalence and intensity of parasite infection

Three to five crabs were removed from the liquid nitrogen at a time and placed under running tap water for a few minutes to hasten thawing. The crabs were then placed on a plastic tray and dissected by removing the carapace and exposing the internal organs. The carapace was broken and examined according to Drach (1939) to ensure that only inter-moult crabs were collected. The digestive gland tissue was removed with a pair of forceps and placed into a glass petri dish resting on a bed of ice. Small amounts of digestive gland were then squashed between two microscope slides and examined under a

dissecting microscope for the presence of trypanorhynch plerocercoids. The squashed tissue, including plerocercoids, was then transferred to another collecting petri dish on ice and the process repeated until the entire digestive gland of the crab had been examined and collected. Trypanorhynch numbers were counted for each individual crab to determine the percentage prevalence and average intensity of infectation.

Enzyme extraction

The squashed digestive gland (including the plerocercoids) for each individual was homogenised for five minutes in 20 ml of chilled extraction buffer (100 mM Tris, 20 mM NaCl, pH 7) using an electric Ultraturrax disperser (IKA works - Germany). The homogenate was centrifuged for 5 minutes at 10 000 rpm and 1 ml aliquots of supernatant transferred to microfuge tubes and stored at –20°C.

Assays

One enzyme unit (U) was defined as the amount of enzyme that catalysed the release of 1 μmole of product per minute and was calculated using the appropriate molar extinction coefficient (ε) in the assay. Specific activity was defined as enzyme activity (U) per mg of digestive gland protein (U mg protein $^{-1}$) and total activity was defined as enzyme activity (U) per digestive gland extract (U digestive gland extract^{-1}). Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as the standard. Chemicals were purchased from Sigma- Aldrich (Castle Hill, New South Wales) and ICN Biomedicals (Aurora, Ohio).

Enzyme assays (trypsin, α-glucosidase, lipase) were performed in duplicate at 37°C and the absorbances read using a TECAN Spectro Rainbow Thermo microplate reader in IWAKI 96 flat bottom-well microplates. Each assay was conducted as detailed below. The linearity of enzyme activities with incubation time and enzyme concentration was checked before conducting assays. All data points are the mean of duplicate assays accounting for the appropriate blanks.

Trypsin

Trypsin was assayed by its amidase acivity using N-α-benzoylarginine-ρ-nitroanalide (BAPNA) dissolved in dimethylformamide (DMF) as substrate. The assay mixture (200 µl) contained a final concentration of 1.25 mM BAPNA (10 µl) in 200 mM Tris, 200 mM NaCL, 10 mM $CaCl_2$, and 0.2% (w/v) polyethylene glycol 6000 (PEG) buffer at pH 7.5. Assays were initiated by the addition of enzyme extract (10 µl) and the release of ρ-nitroanalide measured at A_{400}. Under these assay conditions the extinction coefficient was 9 300 $M^{-1}cm^{-1}$ for ρ-nitroanalide (Stone *et àl.*, 1991).

α-Glucosidase

α-Glucosidase activity was determined using the substrate ρ-nitrophenyl α-D-glucopyranoside. The assay mixture (1ml) contained a final concentration of 20 mM substrate (200 µl) in 100 mM citrate phosphate buffer at pH 7. Assays were initiated with the addition of enzyme extract (100 µl). Aliquots (100 µl) of assay mixture were then removed at time intervals and added to 900 µl of 1 M Na_2CO_3 at pH 11, to terminate the reaction. Liberation of ρ-nitrophenyl was measured at A_{400}. The molar coefficient is

18 300 $M^{-1}cm^{-1}$ for ρ-nitrophenyl at pH >9 (Erlanger *et al.*, 1961).

Lipase

Lipase activity was determined using a modified method of Gjellesvik *et al.* (1992) using 4-nitrophenyl caproate (4-NPC) dissolved in ethanol as a substrate. The assay mixture (200 µl) contained a final concentration of 2.5 mM 4-NPC (5 µl) in 6 mM sodium taurocholate, 500 mM Tris, 100 mM NaCl buffer pH 8.5. Assays were initiated by the addition of enzyme extract (5 µl) and the release of nitrophenol as measured at A_{405}. Under these conditions the molar extinction coefficient was 19 800 $M^{-1}cm^{-1}$ for nitrophenol (Gjellesvik *et al.*, 1992).

Statistics

Pearson-Product Moment Correlation determined the association between host crab size and the number of plerocercoid cysts per host (Dytham, 2003). Regression analyses determined the relationship between the intensity of crab infectation (worms per crab) and specific enzyme activity of trypsin, lipase and α-glucosidase.

RESULTS

Percentage prevalence and mean intensity of plerocercoid infectation were 93.1 and 16.6, respectively. Infectation levels per crab are reported in Table 1. Only two of the 29 crabs were uninfected. Pearson Product-moment correlation showed a significant positive association between the intensity of infectation and crab carapace width ($r^2 = 0.41$, d.f. = 1,27, $P < 0.05$) (Fig.1).

Table 1. Prevalence and intensity of infection of *Carcinus maenas* by *Dollfusiella martini*.

Crab	Carapace Width (mm)	Sex	# of Worms	Crab	Carapace Width (mm)	Sex	# of Worms
1	70	M	32	15	44	F	11
2	72	M	42	16	49	M	0
3	71	M	22	17	58	M	9
4	47	F	0	18	51	M	7
5	61	M	7	19	52	F	10
6	57	M	23	20	64	M	25
7	57	M	24	21	63	M	11
8	65	M	24	22	51	F	24
9	42	M	9	23	50	F	6
10	62	M	21	25	62	M	45
11	63	M	22	26	63	M	16
12	50	F	1	27	59	M	3
13	51	M	25	28	66	M	8
14	40	F	1	29	69	M	37

Prevalence = 93.1 % Average Intensity = 16.6; n = 16

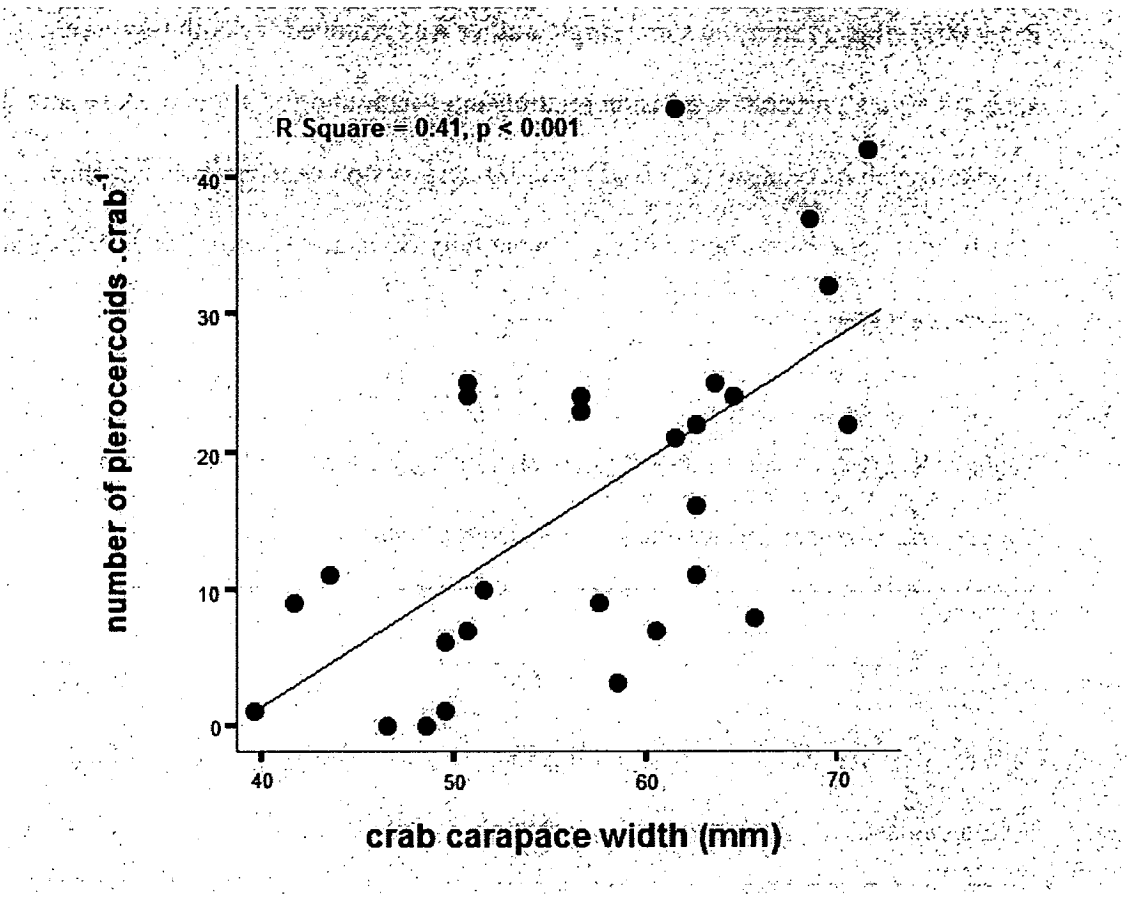

Figure 1. Association between crab size (carapace width mm) and level of infection (number of plerocercoids per crab).

The specific activities of three digestive enzymes were quantified using biochemical assays (trypsin, α-glucosidase, lipase). Specific activity ranged from 0 to 0.0004 units .mg protein $^{-1}$ (trypsin), 0 – 0.0185 units .mg protein $^{-1}$ (α-glucosidase) and 0.0002 – 0.031 units .mg protein $^{-1}$ (lipase).

Regression analysis showed a significant negative relationship between trypsin specific activity and level of trypanorhynch infectation ($r^2 = 0.17$, d.f. = 1,22, $P < 0.05$) (Fig.2) and lipase specific activity and level of trypanorhynch infectation ($r^2 = 0.40$, d.f. = 1,23,

$P < 0.001$) (Fig. 3) after the same single outlier was removed for both regression analyses. No significant regression relationship was found for α-glucosidase activity and trypanorhynch infectation ($P > 0.05$) (Fig. 4). Regression analysis failed to show significant relationships between enzyme activity (total and specific) and sex, for all enzymes assayed.

Table 2. Regression values for a linear relationship between specific enzyme activity and intensity of plerocercoid infectation of *Carcinus maenas* (Linnaeus).

Enzyme	r^2	F	df	p
Trypsin	0.17	4.506	1,22	0.045
Lipase	0.40	15.35	1,23	0.001
α-Glucosidase		2.311	1,25	0.141

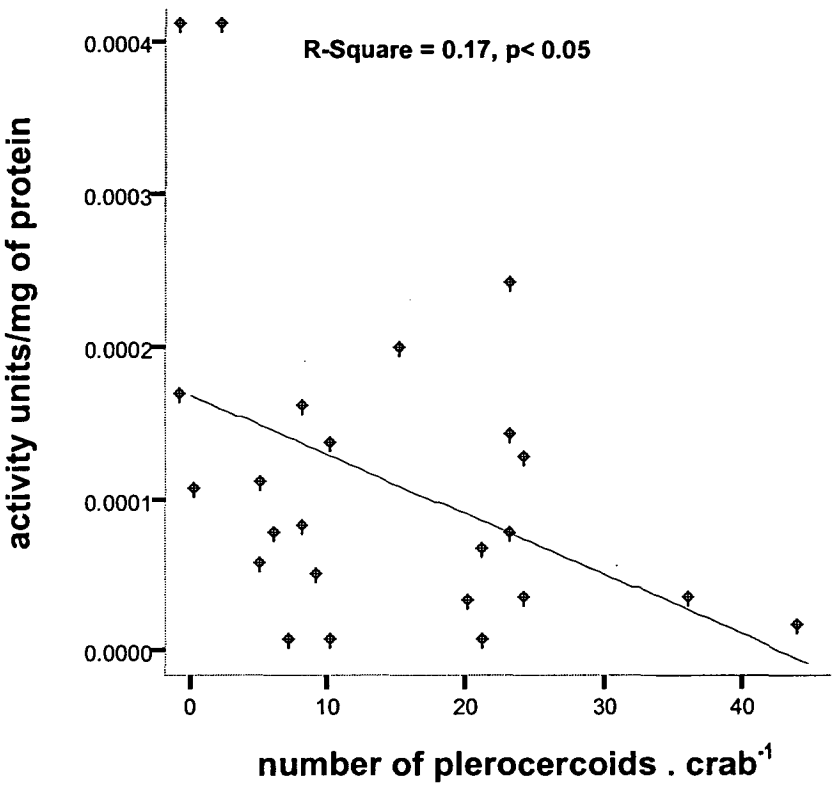

Figure 2. Relationship between trypsin specific activity and intensity of trypanorhynch infection of *C. maenas.*

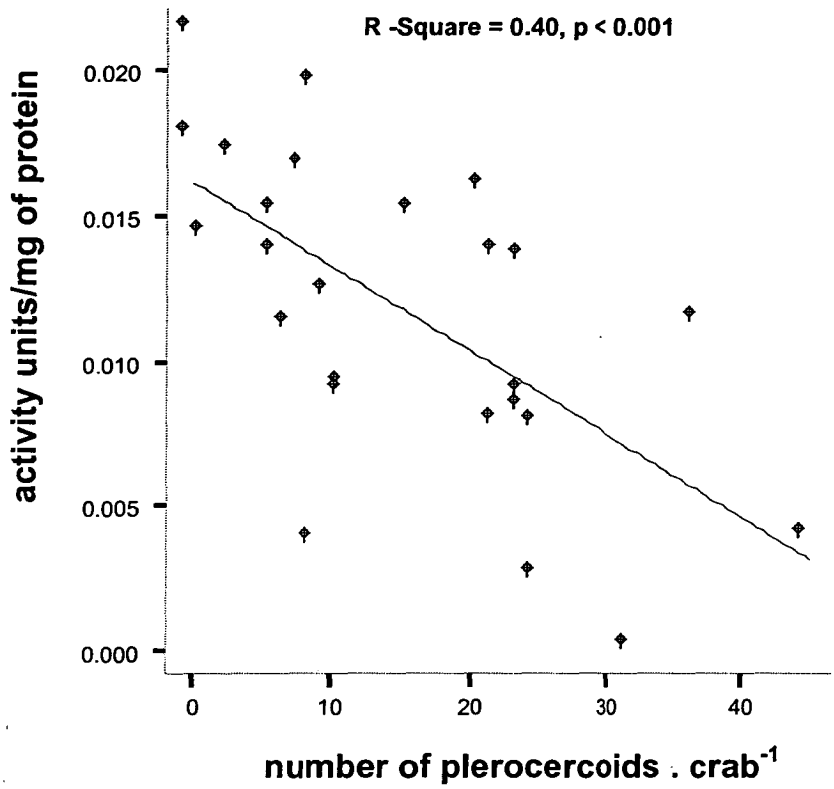

Figure 3. Relationship between lipase specific activity and intensity of trypanorhynch infectation of *C. maenas*.

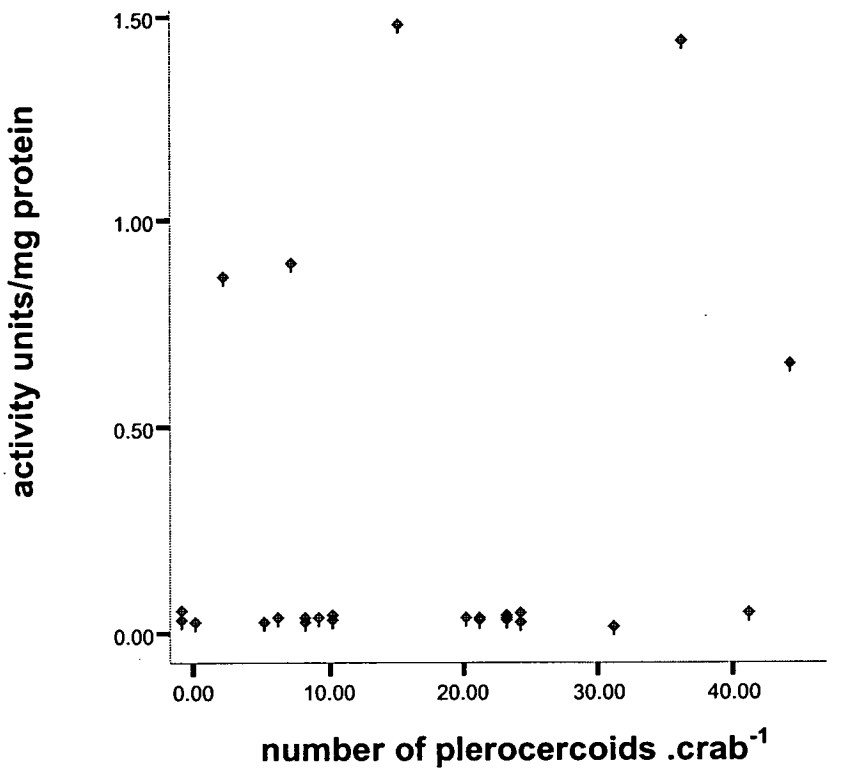

Figure 4. Relationship between α-glucosidase specific activity and intensity of trypanorhynch infection of *C. maenas*.

DISCUSSION

This study has shown that the presence of Trypanorhynch plerocercoids in *C. maenas* may reduce the levels of the digestive enzymes lipase and trypsin produced in the digestive gland. We believe that the physical damage of digestive gland tubules caused by trypanorhynch infectations is the likely explanation for reduced enzyme levels. The epithelial cells of the tubules were transformed from their normal columnar shape into compressed cuboidal and squamous cells due to the pressure exerted by the plerocercoid larvae within the lumen of the tubules. Pressure atrophy of the tubule epithelia frequently occurred in heavy infectations and this resulted in the destruction of all differentiated epithelial cell types. These changes were often accompanied by pronounced inflammatory changes in the interstitium and the formation of granulomas around infected tubules (Gurney *et al.*, 2004). We propose that the reduced specific activity of trypsin and lipase is due to the diminished number of functioning fibrillar (F) cells which synthesize and secrete digestive enzymes (Johnston and Yellowlees, 1998, Hopkin and Nott, 1980). Furthermore, the reduced specific activities of lipase may also be due to diminished numbers of resorptive (R) cells, which have been shown to contain lipases in the digestive gland of the European lobster, *Homarus gammarus* (Linnaeus, 1758) (Barker and Gibson, 1977). Total protease, lipase and amylase activities generally increase with the increasing number and length of the digestive gland tubules in developing lobster larvae (Biesiot and Capuzzo, 1990.) It is reasonable, therefore, to expect the destruction of digestive gland cells to result in decreased in enzyme synthesis as measured by specific enzyme activity.

Host exploitation theory suggests that selective pressures ensure that the level of costs due to increased host mortality equal the benefits of reproductive success for the parasite (Anderson and May, 1982). A green crab with low digestive enzyme activity, resulting from high plerocercoid loads, may suffer the cost of reduced food assimilation. This, in turn, may force the crab to forage longer for more food in an attempt to restore its energy balance. Extended foraging may represent parasite mediated behaviour which would ensure that the parasitised host is exposed for longer periods to predation by final hosts, thereby completing the parasite's life-cycle. Here the nutritional cost to the host represents a reproductive benefit to the parasite. There are many examples of parasite induced behavioural changes in crustaceans, particularly in relation to acanthocephalans (Bethel and Holmes, 1977, Haye and Ojeda, 1998, Hecthel *et al.*, 1993). However, it is not possible to attribute parasite induced adaptive host behaviour without rigorous investigation (Poulin, 2000) and we raise this as a possibility for further study.

There was no significant relationship between α-glucosidase specific activity and trypanorhynch infectation. Green crabs are omnivorous, feeding mainly on bivalves, small crustaceans and some algae. Gut content analyses of green crabs reveal that algae constitutes only a small fraction of their usual diet (Grosholz and Ruiz, 1996; Mackinnon, 1997; Elner, 1981) and this might explain the large number of zero readings for α-glucosidase. Other researchers have shown α-glucosidase to be the lowest carbohydrase activity reported in *Cancer borealis* (Stimpson, 1859) (see Brun and Wojtowicz, 1976). However, a few crabs in our study recorded high activity levels for this enzyme but there

was no discernable pattern. We consider it more likely that a laboratory artifact explains the unusual distribution of results for α-glucosidase activity.

The specific activities for trypsin and lipase were low (trypsin: 0 – 0.0004 units .mg protein $^{-1}$ and lipase: 0.0002 – 0.031 units .mg protein $^{-1}$). Trypsin has been measured in *C. maenas* at ~ 0.2 units.mg protein $^{-1}$ (Johnson and Freeman, 2005) and lipase measured at 0.371 units. mg protein $^{-1}$ in the spiny lobster *Jasus edwardsii* (Hutton, 1875) (Johnston, 2003). The specific activity of trypsin in the prawn, *Penaeus japonicus* (Bate, 1888), has been measured at 0.057 and 0.73 units .mg protein $^{-1}$ (Galgani, 1984, Maugle, 1982). Nevertheless, it is difficult to compare the results of these analyses against our results because of the effects of differences in diet, time of capture and time of last meal between the crustaceans from the various studies.

It is possible that the plerocercoids, left in the assayed digestive gland tissue, may have contributed to the inhibition of trypsin and lipase activity of the prepared digestive gland extract. A number of researchers have demonstrated the inhibition of proteolytic enzymes which are in contact with intact tapeworms (Schroeder *et al.*, 1981). Intact adult cestodes, *Hymenolepis diminuta* (Rudolphi, 1819), in direct contact with trypsin produced significant trypsin inhibition when using azoalbumin as the substrate. However, there was no inactivation of trypsin when assays used synthetic substrates (Schroeder *et al.*, 1981). We used one of the same synthetic substrates (BAPNA) and we therefore do not expect trypsin inactivation to have occurred in our assays.

Although our enzyme activity levels are lower than expected, the inverse relationship between enzyme activity and level of plerocercoid infectation remains for both lipase and trypsin, demonstrating a quantifiable physiological consequence of parasitism by plerocercoids.

The positive correlation between intensity of infectation and host size, observed in this study, is a well-described parasitological phenomenon which has been noted in fish and crustacea (Rigby, 1996, Poulin, 2000, Shields, 2000,). This relationship is not altogether surprising, considering a larger host is generally an older animal which has had longer time to accumulate parasites. Furthermore, a larger host has greater external surface area for ectoparasites and internal surface area or volume for endoparasites.

While enzyme activities were standardized against crab size, by measuring specific activity instead of total activity, it may be argued that decreased specific activities of trypsin and lipase could be related to crab age. The effects of age upon digestion might include, for example, degeneration of the digestive gland structure and function, a shift in diet and possibly associated flora of the digestive gland and gut or a change in metabolic rate. Degenerative changes in the digestive glands of *C. maenas* might be expected to occur in the terminal moult of the crabs' life – a period of senility and possible digestive gland ageing (Crothers, 1967).

None of the collected crabs were heavily encrusted with epizooans, suggesting they had not reached terminal moult. Furthermore, Male *C. maenas* attain sexual maturity at 25–

30 mm and females between 15 and 31 mm (Crothers, 1967). Our crabs were well within the adult range and were aged between 1.5 – 2 years, as suggested by growth data from invasive green crab populations in Oregon, USA (Kalin and Yamada, 2000). We consider it unlikely that there was a sufficiently wide age gap in our collected crabs to demonstrate any possible age related effects on digestion. However, to conclusively rule out an age effect, the specific enzyme activities of infected crabs from this trial needed to be compared against the specific enzyme activities of uninfected crabs of various ages. Finding a control group of uninfected crabs from a location of zero prevalence would pose problems of possible dietary differences arising from the geographic separation of the uninfected and infected crabs. This may be alleviated by feeding the same diet before sampling.

In conclusion, regression analysis showed a significant negative relationship between the intensity of plerocercoid infectation and the specific activity of trypsin and lipase in the digestive gland of green crabs. This suggests that encysted plerocercoids have an adverse effect on the digestive enzyme physiology of the green crab host. We believe that this reduction in activity is due to tissue damage of enzyme producing F cells caused by plerocercoid infectation. The negligible effect on the specific activity of α-glucosidase remains unclear and requires further investigation.

Plerocercoids of *D. martini* and melanised granules were localized within a specific area of the digestive gland, in the antero-dorsal segment of the digestive gland, near the juncture of the cardiac stomach and midgut intestine. Because we believe reduced

enzyme activity to be a result of physical tubule destruction, future digestive enzyme assays of the infected tissue as opposed to the entire tissue may strengthen the negative linear relationships between enzyme activity and level of plerocercoid infectation.

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CHAPTER 5

SACCULINA NECTOCARCINI A NEW SPECIES OF RHIZOCEPHALAN (CIRRIPEDIA: RHIZOCEPHALA) PARASITISING THE RED ROCK CRAB *NECTOCARCINUS INTEGRIFRONS* (DECAPODA: BRACHYURA: PORTUNIDAE)

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SACCULINA NECTOCARCINI, A NEW SPECIES OF RHIZOCEPHALAN (CIRRIPEDIA: RHIZOCEPHALA) PARASITISING THE RED ROCK CRAB *NECTOCARCINUS INTEGRIFRONS* (DECAPODA: BRACHYURA: PORTUNIDAE)

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ABSTRACT: The parasitic barnacles, rhizocephala, are a little known group within Australia with only about seven described species from a coastline stretching approximately 59763 km. This study describes a new species of rhizocephala: *Sacculina nectocarcini*. The new species is described as a separate species based on a unique combination of features pertaining to the structure of the mantle papillae, the retinaculae and the male receptacles. Biological notes, prevalence and intensity of infection are reported for this rhizocephalan, infecting red rock crabs, *Nectocarcinus integrifrons* (Latreille, 1825) collected from Western Port, Victoria, Australia.

Key Words: retinaculae, externa, parasitic castration, cyprid, Rhizocephala, parasitic barnacle

INTRODUCTION

Nectocarcinus integrifrons (Latreille, 1825) is a common low intertidal and subtidal portunid crab endemic to Australia's temperate coastline, from Port Jackson, New South Wales, to Victoria, Tasmania, South Australia and Western Australia, (N to Fremantle) (Poore 2004). Specimens collected from Western Port, Victoria in 1997, were parasitised by an unknown rhizocephalan. This barnacle parasite may have been first recorded by Haswell (1888) who recognised the 'firm but soft brown body' beneath the abdomen of *N. integrifrons* and *Thalamita sima* (Milne Edwards,1834), collected from Port Jackson, as a rhizocephalan of the genus *Sacculina*. Boschma (1955) speculated that Haswell's record may have been *Sacculina angulata* (Van Kampen & Boschma, 1925). A photograph of *N. integrifrons* in Hale (1927), apparently sacculinized, provides the only other reference to this parasite. An extensive crab survey of Port Phillip Bay, 23 kilometres to the west of Western Port, failed to discover any sacculinized *N. integrifrons* (Griffin and Yaldwyn, 1971), and these parasites were not detected from *N. integrifrons* collected from Queenscliff, Port Phillip Bay (n=14) or Humbug Point (n = 16) and Stieglitz (n = 17) in Georges Bay on the east coast of Tasmania (Kuris and Gurney unpublished data).

Since Haswell's discovery of the unnamed 'sacculina', seven sacculinid species have been identified from Australian waters. Phillips (1978) lists *Sacculina duracina* (Boschma, 1933) from *Parthenope longimanus* (Linnaeus, 1758) at Port Molle, Queensland and *Sacculina granifera* (Boschma, 1973) from *Portunus pelagicus* (Linnaeus, 1758) at Moreton Bay, Queensland and describes a further three species, all

from Moreton Bay, including *Sacculina amplituba* (Phillips, 1978) from *Matuta granulosa* (Miers, 1877), *Heterosaccus lunatus* (Phillips, 1978) from *Charybdis callianassa* (Herbst, 1801) and *Heterosaccus multilacinencis* (Phillips, 1978) from *Charybdis truncata* (Fabricius, 1798). Boschma (1957) also described *Loxothylacus spinulosus* (Boschma, 1928) from *Pilumnopeus serratifrons* (Kinahan, 1856) off Sydney, New South Wales. The most recently discovered species is *Loxothylacus ihlei* (Boschma, 1949) from the mud crab *Scylla serrata* (Forskål, 1775) in northern Australia (Knuckey *et al.*, 1995).

Here we describe the sacculinid parasitising *N. integrifrons* and provide biological observations on the specimens collected from Western Port, Victoria, Australia in May 1997, October 1997 and April 1998.

MATERIALS AND METHODS

N. integrifrons were caught in the North Arm of Western Port, Victoria, Australia (Lat. 38° 3′– 38° 4′ S and Long. 145° 1′–145° 2′ E) using baited crab traps deployed overnight (Fig. 1). The sampling occurred in early May 1997, as part of an extensive survey of the area for parasites of intertidal and sub-tidal crabs. Two subsequent trips in October 1997 and Late April 1998 were made to Sandstone I., Western Port to collect live specimens of sacculinised *N. integrifrons* for laboratory studies. On all occasions traps were set at depths between 1 to 4 m.

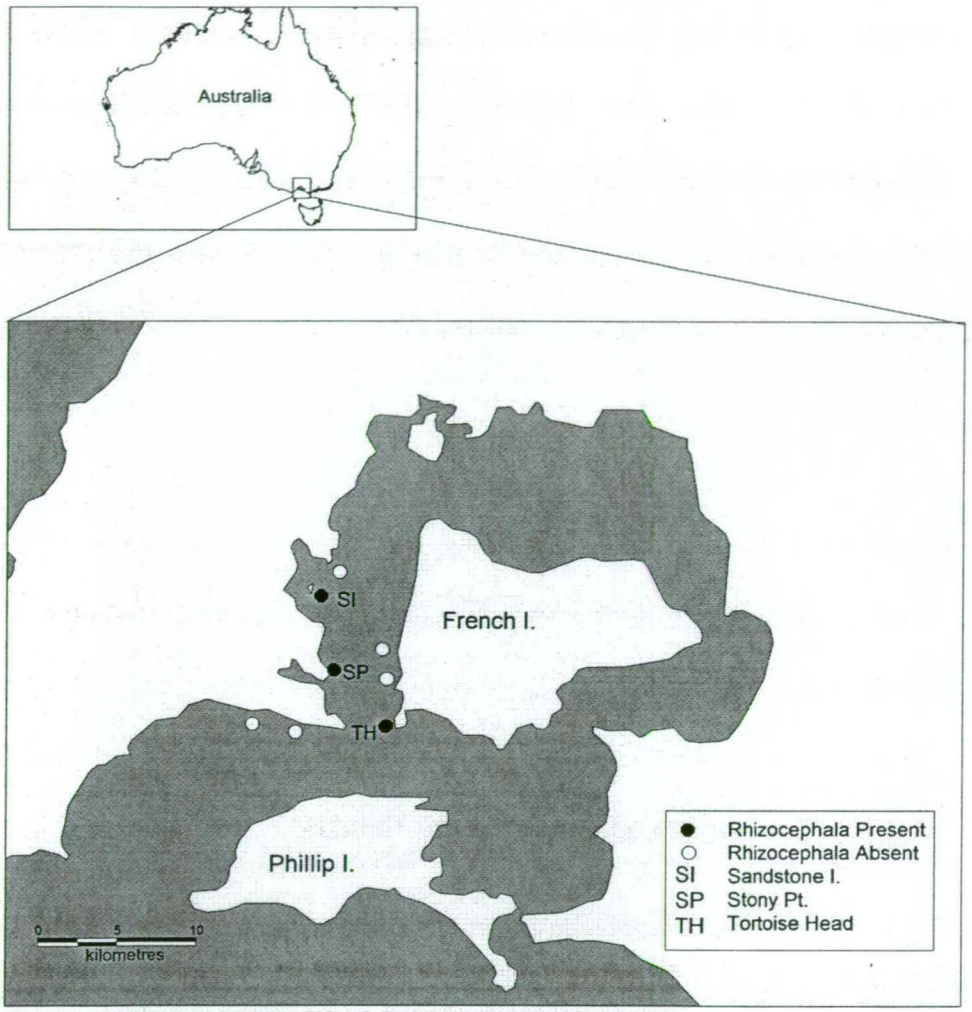

Figure 1. sampling sites in Western Port Victoria.

Trapped crabs were examined to determine: (1) sex and size (carapace width, to the nearest mm); (2) crab moult stage (according to Drach, 1939); (3) the presence of a rhizocephalan external brood chamber (externa) and the effect of modification of secondary sexual characters (chiefly the measurement of abdominal breadth in mm); (4) the stage of development of the externa; (5) presence of externa scars; (6) presence of rhizocephalan internal ramifying processes (interna) where no externa was found. The prevalence of infection was determined by the presence of externa and interna during the first two collections (May, 1997 and October 1997). The presence of externa only was used to determine prevalence in the third collection (April, 1998) (Tables 1 and 2).

Table 1 Prevalence of infection of *N. integrifrons* by *S. nectocarcini* sp. nov. in Western Port, Victoria.

Location	n	% Prevalence
Chilcott Rocks	16	0
Hastings Bight	6	0
Sandstone Island	23	56.5
Sandstone I. (October 1997)	24	33.3 (interna only)
Sandstone I. (April 1998)	25	56
Stony Pt.	59	32.2
Tortoise Head	15	13.3
Somers	1	0
Merricks Beach	1	0

Table 2. Prevalence of infection of *N. integrifrons* by *S. nectocarcini* sp. nov. according to host sex.

	Locations							Total
	SI	SP	TH	HB	CR	MB	S	
Total ♂	9	35	10	4	14	1	1	74
♂ with externa	3	7	1					11
(% prevalence)	33.3	18.9	10					14.9
♂ with interna	2	2						4
(% prevalence)	22.2	5.7						5.4
	55.5	24.6						20.3
Total ♀	14	24*	5	2	2			47
♀with externa	2	8						10
(% prevalence)	14.3	33.3						21.3
♀ with interna	6	2	1					9
(% prevalence)	42.9	8.7	10					19.1
	57.1	43.5	10					40.4

* Total includes single female with scar.

Key: SI – Sandstone Island
SP – Stony Point
TH – Tortoise Head
HB – Hastings Bight
CR – Chilcott Rocks
MB – Merricks Beach
S – Somers

Externae were categorised as virgin (V), immature (I) or mature, containing early developing embryos (ME), or advanced, eyed embryos or nauplii (MA) (Table 3). Virgin externae are very small and have not yet received males in their seminal receptacles, immature externas do not have brood in their mantle cavities (Høeg and Lützen, 1995).

Table 3. Developmental stages of *S. nectocarcini* sp. nov. infecting *N. integrifrons*.

	Western Port all locations combined (May 1997)	Western Port Sandstone I. (October 1997)
MA	1	
ME	17	
I		
V	1	
Interna only	14	8
Total *N. integrifrons* examined	145	24

Key: MA – Mature externa with advanced embryos
ME – Mature externa early developing embryos
I – Immature externa
V – Virgin externa

Specimens were fixed in Bouin's fluid or alcohol, formalin, acetic acid fixative (AFA) and transferred to 70% ethanol. The tissue was processed routinely for paraffin wax infiltration and sections (6 μm) were stained with haematoxylin and eosin (H & E). Fixed sections of cuticle were transferred into distilled water, postfixed in 2% OsO_4 for 1 hr, rinsed in distilled water for several hours, dehydrated in a graded series of ethanol, ethanol + acetone, and 100% acetone solutions, critical point dried in CO_2 and mounted for observations. Due to the relatively large size of the mounted samples and their complicated surface pattern, the specimens were treated for 8–10 minutes is a sputter-coater, which is longer than usually recommended. A JEOL JSM 6335-F microscope at the Zoological Museum, University of Copenhagen was used for the observations.

Externa size is described in mm by length (L), the distance between mantle opening and stalk base, height (H), the greatest distance between dorsal and ventral margins and width (W), greatest distance between lateral margins (Huang and Lützen 1998; Smith, 1906) (Fig.2).

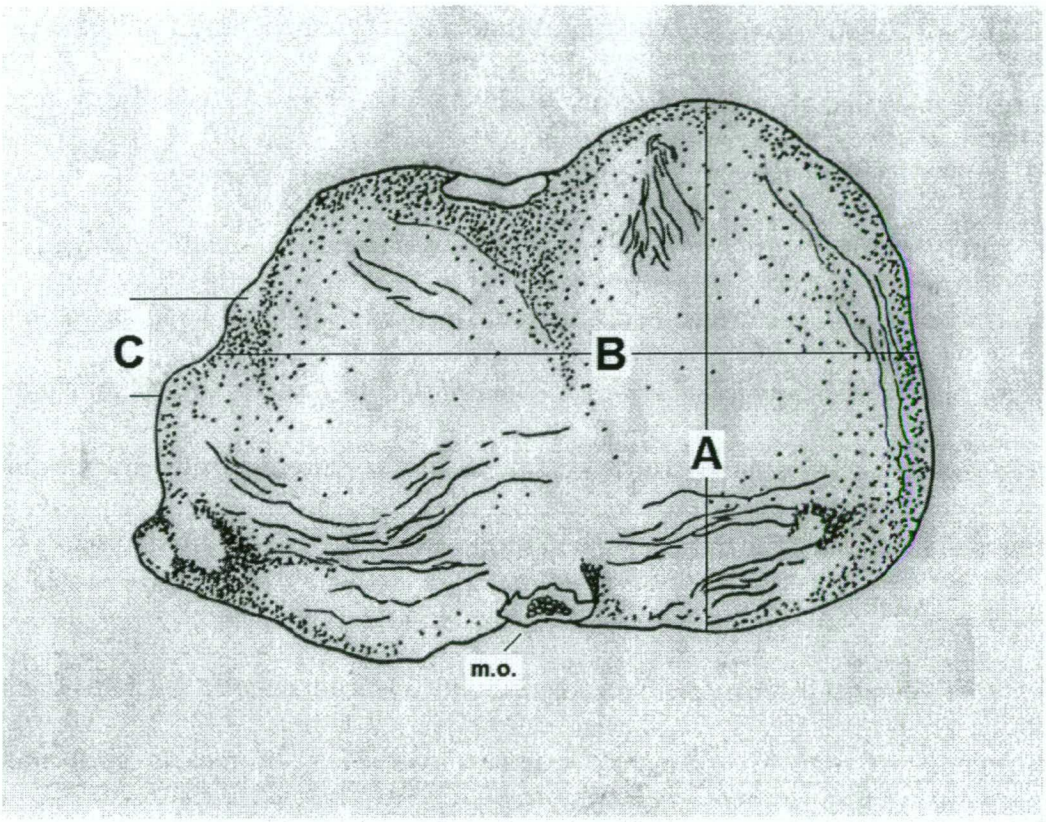

Figure 2. *Sacculina nectocarcini* entire externa, left side. A, B and C are the anterior to posterior, dorsal to ventral and left to right dimensions respectively; m.o., mantle opening.

RESULTS

Sacculina nectocarcini sp. nov.

Materials

We examined three specimens from the red swimmer crab, *Nectocarcinus integrifrons* (Latreille, 1825); Tasmanian Museum and Art Gallery; ref. nos. G5467, G5468 and G4029. **Holotype:** G5467 (from Stoney Point, Western Port, Victoria, May 1997: externa attached to the modified abdominal flap of a male crab) is deposited in the Tasmanian Museum and Art Gallery (Hobart, Tasmania). **Paratype:** G5468 (from Stoney Point, Western Port, Victoria, April 1997: detached externae) deposited in the Zoological Museum, University of Copenhagen (Copenhagen, Denmark). G4029 was dissected and prepared for SEM. The holotype is 11 × 19 × 8 mm in size: the stalk is very short, about 1.5 mm in diameter. The paratype is 13 × 16 × 12 mm in size; the stalk is 2 mm in diameter and 4.5 mm long. The third examined specimen is 7.5 × 16 × 5.5 mm in size.

Other material: Virginal externae were rectangular with a smooth cuticle. We noted sizes ranging from 2 – 6 mm W. A mature externa (15 x 27 x 11 mm in size) was photographed (Fig. 3) and subsequently sectioned and stained with H & E (Fig. 8).

Colouration

Preserved externae were light yellow-brown. In live specimens colour ranged from light yellow, in smaller, presumably younger, specimens, to light to medium brown in larger specimens. Externae with larvae became increasingly deep purple as the larvae developed eyespots. Virgin externas were light yellow.

Position on host

Externae emerged from the ventral cuticle of the second or third abdominal segment.

Diagnosis and description

The mature externa is brown-coloured and almost symmetrical, wider than long, with the dorsal side slightly larger than the ventral one. The shape varies from more or less rounded to trapezoidal, with the anterior region significantly larger than the posterior one. The anterior margin is almost straight and continues into a pair of conspicuous dorsal and ventral protuberances (the 'shoulders'). The latter are pointed or rounded; the ventral shoulder is usually larger than the dorsal one. The posterior margin is concave and may continue into a second pair of 'shoulders', which, when present, are much smaller than those of the anterior margin and directed backwards. The stalk is rather thin and, in some specimens, also rather long: it arises from the center of the posterior margin. The stalk has no visible annuli and is slightly flared at both ends where it attaches to the host at one end and to the externa at the other. The mantle opening is located at the center of the anterior margin. It is somewhat shifted to the left side and slightly elevated above the surface of the externa.

The cuticle surface becomes increasingly wrinkled in mature specimens, with wrinkles running parallel along the anterior and dorsal and ventral margins. Older specimens may have worn or abraded cuticular surfaces (Fig. 3). The cuticle is very thick and rigid and consists of numerous layers. Below it extends a thick muscle layer with fibres running in different directions (Fig. 4). The external surface is densely covered with simple

spiniform papillae. They are 6–9 μm long, about 1 μm wide at the base and arranged in groups of 3–12 papillae each so that their basal parts are fused to form a common base (Fig. 5). The internal surface of the mantle is smooth or, in some areas, covered with minute setae and it bears a few scattered, but large retinaculae. The retinaculae consist of a cylindrical basal part, 30–65 μm long and 20–35 μm in diameter (sometimes swollen) and with 11–25 barbed, and 14–22 μm long spindles (Fig 6). The subjacent layer of cuticle has a very peculiar structure. It consists of a loose fibrous material that forms irregular elevations separated by narrow grooves. In some areas retinaculae can be seen as groups of smooth spindles located at the bottom of oval depressions in the cuticle (Fig. 7).

The mesentery is complete, thin and extends from the stalk to the mantle opening. The visceral sac is asymmetrical, with the dorsal side significantly larger than the ventral one. There is a deep groove on the right side of the sac, running from the base of the stalk to the mantle opening and a corresponding ridge extends on the left side. The colleteric glands are located in the centre of the left and right surfaces of the visceral sac. They consist of numerous (ca. 100–120) canals, visible in longitudinal sections through the central part of the gland. The canals are arranged in 6–7 rows (Fig. 8) and villiform projections (5–7 μm L) line their inner surface. The male receptacles are embedded in the visceral mass close to the stalk; they are directed dorso-ventrally and closely applied to each other, but not fused. The left receptacle is better developed, straight and cylindrical in shape; it has very thick walls with smooth inner surface and a wide lumen. The right receptacle is much smaller, also straight, strongly compressed laterally and crescent-

shaped in transverse section, so that it has almost no lumen. Whether this difference is caused by one receptacle being invaded by a male trichogon and the other being sterile is unknown as only one specimen has been sectioned. The receptacles gradually pass into short, somewhat tortuous ducts, furnished with numerous distinct ridges along the inner surface. The right duct is shorter than the left. The receptacle ducts are almost as wide as the receptacles, and they exit into mantle cavity at the level of stalk.

Figure 3

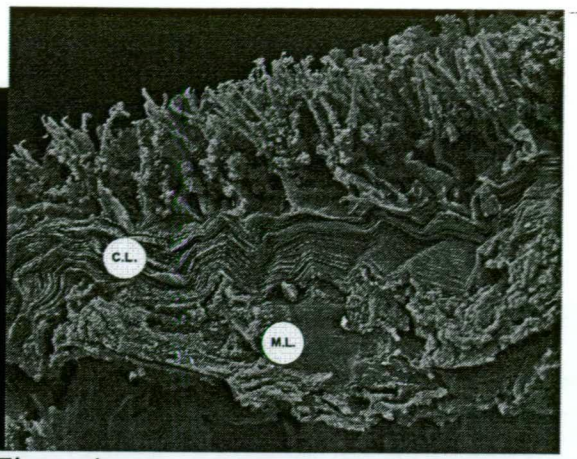

Figure 4

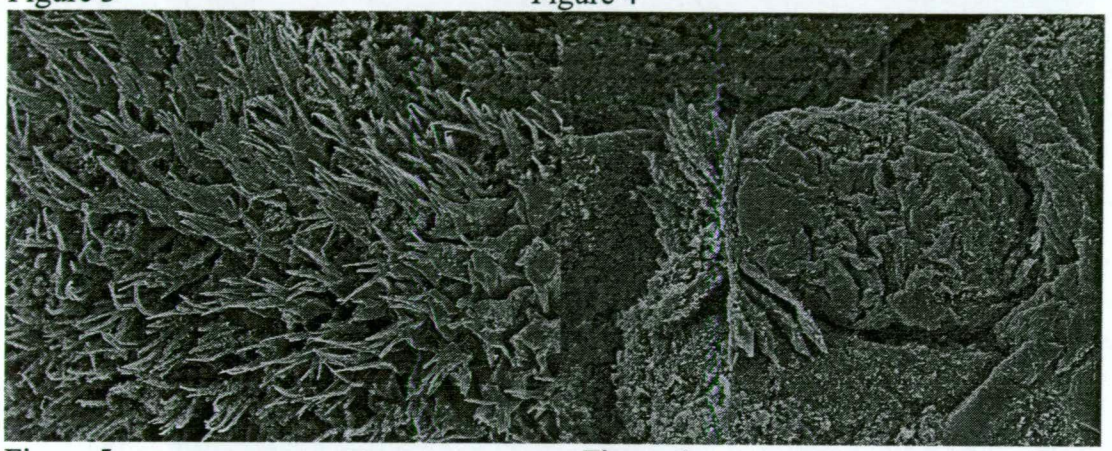

Figure 5

Figure 6

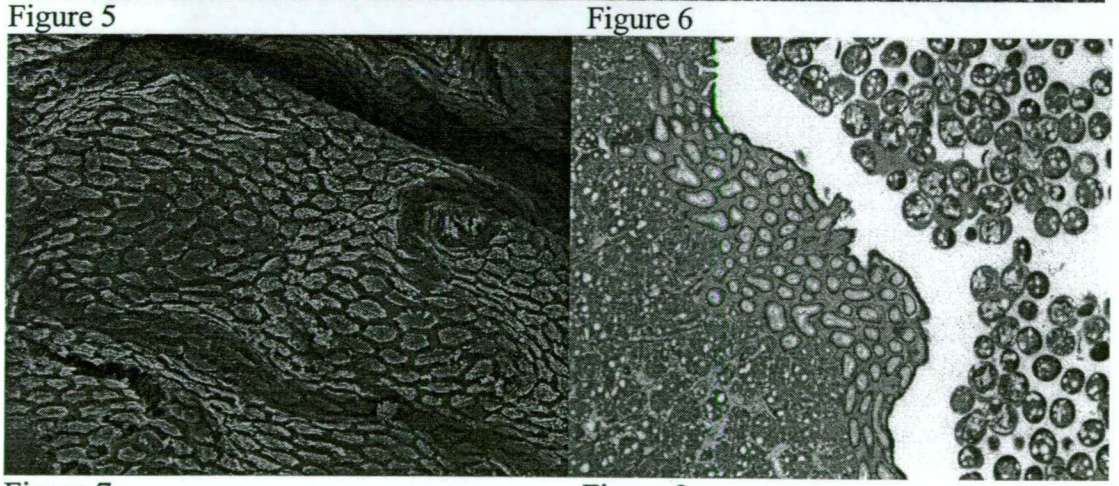

Figure 7

Figure 8

Figure 3. *Sacculina nectocarcini* sp. nov. Figure 4. Cuticle consisting of numerous layers, C.L. cuticle layers, M.L. muscle layer. Figure 5. Simple spiniform papillae on external cuticle surface. Figure 6. Group of retinaculae on inner mantle surface. Figure 7. Group of retinaculae located in oval depression on inner mantle surface. Figure 8. Colleteric tubules (200 x).

Remarks

The new species differs from *Sacculina carcini* (Thompson, 1835) in the structure of the mantle papillae, the retinaculae and the male receptacles. Ideally, any new species should be described in terms of supposed automorphic traits compared to other closely related species. In the Rhizocephala this is not yet possible using morphological characters only. The combination of characters seen in the new species is not known from any described rhizocephalan, and for the present this, and an analysis of the mitochondrial DNA (CO1 region) (Gurney *et al.* in press) and ribosomal RNA (ITS1 region) (Murphy and Goggin, 2000) which distinguished this parasite from *Sacculina granifera* (Boschma, 1973), *S. oblonga* (Lützen and Yamaguchi, 1999) and *S. carcini*, must suffice to separate it from other species.

Etymology

The name is derived from its known host *Nectocarcinus integrifrons* and is consistent with the naming convention used for the closely related rhizocephalan *Sacculina carcini* which parasitises *Carcinus maenas* (Linnaeus, 1758), a portunid crab from the same family (Carcininae) as *N. integrifrons*.

Effects on Nectocarcinus integrifrons

The parasite modified the morphology of the male abdomen by broadening it so that it more closely resembled the female abdomen. However, modification is not complete and parasitised males remained distinguishable from females because the male pleopods were not modified or fused as a consequence of infection. This contrasts with the observations

of Haswell (1888) who described extensive modification to the abdominal appendages of sacculinised male and female *N. integrifrons*. The parasitised male abdomen loses its concave lateral outline, becoming flattened and slightly convex as described for *Portunus pelagicus* (Linnaeus, 1758) parasitised by *S. granifera* (cf. Weng, 1987). We did not find any evidence for hyperfeminisation (an abnormally wide abdomen) of parasitised female *N. integrifrons.*

No ovaries nor testes were present in crabs with externas. Degenerating ovaries were observed in female crabs infected with an interna.

Prevalence and Distribution

The first survey of Western Port (May 1997), covered 7 sampling locations within the Western Channel and North Arm (Fig. 1). *Sacculina nectocarcini* was common at three of the seven locations with prevalences ranging from 13 to 57% (Table 1.) Of the 33 parasitised specimens from the May collection, 60% had an externa with the remaining 40% having only interna. The highest prevalence of infected *N. integrfrons* (56.5%) came from waters 100 to 200 m off the south-east coast of Sandstone Island. Two subsequent collections from Sandstone I. (October 1997, April 1998) revealed prevalences of 33.3% (interna only) and 56% (externa only) respectively.

The prevalence of *S. nectocarcini* did not differ between the sexes for crabs from Sandstone Island and Tortoise Head, but female crabs from Stony Point had nearly double the percentage prevalence of males from the same location (Table 2),

Nectocarcinus integrifrons is of marginal commercial value and is not heavily fished. This may, in part, explain the paucity of reports of rhizocephalan infections for this crab. Variable spatial and temporal prevalences are common features for many rhizocephalans (Heath, 1971, Hochberg *et al.* 1992, Hines *et al.* 1997). Despite its common presence in Western Port, this body of water is little studied compared with its neighbouring bay to the west, Port Phillip Bay, and this may also have contributed to its lack of detection.

Biology

The seasonal life cycle of this rhizocephalan is unknown but it may well follow the life cycle elucidated for *Sacculina carcini* in the northern hemisphere (Høeg and Lützen, 1995). That is, small crabs are infected in the summer or autumn with female nauplii released from mature externas. The interna develops within the infected crab over 9 months. The virgin externas emerge in late spring and are fertilised in summer to mature and produce maximal broods in autumn. We presume a similar life-cycle for this rhizocephalan based on our three collections which revealed large numbers of mature externas in autumn (May 1997 and April 1998) and the absence of externas but presence of internas in spring (October 1997). This seasonal pattern of infection may be the reverse of that described for *S. granifera* infecting *P. pelagicus* in Moreton Bay, Queensland (Lat. 27°– 28° S and Long. 153°–153.25° E) (Sumpton *et al.* 1994) and Mornington Island in the Gulf of Carpentaria (Lat. 11°–17° S and Long. 136°–142° E) where maximum infection, as evidenced by externas, occurred during summer. However, because we did not sample *N. integrifrons* during the summer, we are unable to confidently propose this seasonal pattern.

Three separate attempts were made to hatch nauplius larvae and rear subsequent cypris larvae from infected *N. integrifrons* in recirculating marine aquaria with water temperature maintained at 17°C. In each attempt either eggs or eyed nauplli were aborted by the externa with the host crab usually dying shortly after. Development, from egg to eyed larvae, took 3 – 4 weeks, at which point larvae were aborted and the externa either withered and dropped off, leaving a black scar on the cuticle of the host, or began brooding another batch of eggs. *N. integrifrons* were difficult to keep alive in aquarium conditions and no fully developed cypris larvae could be collected for morphological description because of larval putrifaction in the dying externa.

Two double infections were recorded; each consisted of a large and small externa. The differences in externa size suggesting that the larger externa was the first to be implanted with a trichogon initiating immediate growth (Høeg and Ritchie, 1985). Embryonic development was asynchronous within the externae of double infections.

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CHAPTER 6

POPULATION STRUCTURE IN THE PARASITIC CIRRIPEDE *SACCULINA CARCINI* AS SUGGESTED BY HAPLOTYPE DIFFERENCES IN THE MITOCHONDRIAL CYTOCHROME OXIDASE GENE (COI)

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POPULATION STRUCTURE IN THE PARASITIC CIRRIPEDE *SACCULINA CARCINI* IMPLIED BY HAPLOTYPE DIFFERENCES IN THE MITOCHONDRIAL CYTOCHROME OXIDASE GENE (COI)

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ABSTRACT: Nucleotide sequence data of the mitochondrial cytochrome oxidase I (COI) gene, were compared for 17 specimens of *Sacculina carcini* (Thompson 1836) parasitising three portunid hosts in order to assess population structure in a poorly understood species for which there are few morphological characters on which to base taxonomic analysis. *S. carcini* parsitising *Carcinus maenas* (Linnaeus, 1758) (from Sweden, England and Denmark) were compared with *S. carcini* parasitising *Liocarcinus marmoreus* (Leach, 1814) (from Ireland), and *Liocarcinus holsatus* (Fabricius, 1798) (from Wales). Specimens of three congeneric sacculinid species and one confamilial species, were included in the comparison as outgroups. The comparison showed geographically consistent sequence differences among sampled sites, high levels of similarity within sites, very large differences between species, and greater resolution of spatial population structure than previously reported (Murphy and Goggin, 2000). The data confirm that specimens of *S.carcini* from different hosts and different regions are all the same species, and that analysis of the COI gene sequence could be a useful method for resolving population genetics and taxonomy of rhizocephelans.

Key Words: Sacculina, rhizocephala, cytochrome oxidase, COI, host specificity, population structure, parasitic barnacle, European green crab

INTRODUCTION

Sacculinids are parasitic castrators of decapods, internally parasitising both sexes and destroying the gonads of their host (Høeg and Lützen 1995). *Sacculina carcini* (Thompson, 1836), has been recorded from twelve crab species in six families (Høeg and Lützen, 1985). This host diversity has led to speculation that the nominal *S. carcini* is a complex of rhizocephalan species, which are difficult to separate due to the paucity of morphological features on which to base an analysis (Høeg and Lützen, 1995). Recent suggestions that *S. carcini* might prove useful as a biological control agent against invasive populations of one of its major host species, the European shore crab *Carcinus maenas* (Linnaeus, 1758), depend critically on the degree of host specificity by the parasite (and hence the risk the parasite, once introduced, will attack desirable native species) (Thresher *et al.*, 2000).

Molecular techniques provide new options for assessing the taxonomy and population structure of groups like the sacculinids. Glenner *et al.* (2003), for example, recently proposed the erection of a new sacculinid genus, *Polyascus*, based on analysis of the nuclear ribosomal gene (18s nrDNA) and the mitochondrial cytochrome oxidase I (COI) gene. Murphy and Goggin (2000) examined the internal transcribed spacer 1 (ITS1) and the small subunit (SSU) ribosomal RNA from *S. carcini* parasitising three species of host [*C. maenas, Liocarcinus marmoreus* (Leach, 1814), and *Liocarcinus holsatus* (Fabricius, 1798)]. They concluded that all specimens were likely conspecific, and found little indication of population structuring (Murphy and Goggin, 2000).

The cytochrome oxidase I (COI) gene has been proposed as the basis for the "barcoding of life" project (Hebert *et al.*, 2003), and potentially allows for a finer resolution of population structure than would be possible using the ITS1 or SSU regions. This is because more rapidly evolving mitochondrial genes generally allow relationships to be inferred among groups with recently linked ancestries rather than the more slowly evolving nuclear rRNA which is better suited to resolving relationships among groups with long histories of evolutionary divergence (Remegio and Hebert, 2003). We tested this potential, in order to resolve the host specificity of *S. carcini*, by comparing COI sequences among specimens of nominal *S. carcini* from different regions and hosts, and by comparing the range of variation within the nominal *S. carcini* with sequence data from other sacculinid species.

MATERIALS AND METHODS

DNA was extracted from the externae of *S. carcini* parasitising *C. maenas* from Plymouth, England (n = 4), Nykobing, Denmark (n = 6), and Ellösfjörden, Sweden (n = 4), as well as from putative *S. carcini* parasitising *L. marmoreus* from Coningbeg Rock, Ireland (n = 2) and *L. holsatus* from Anglesey, Wales (n = 1) (Figure 1). DNA was also extracted from a second group of rhizocephalan externae including *Sacculina granifera* (Boschma, 1973), parasitising *Portunus pelagicus* (Linnaeus, 1758) in Moreton Bay, Australia (n = 2), *Heterosaccus lunatus* (Phillips, 1978) parasitising *P. pelagicus* in Moreton Bay, Australia (n = 1), *Sacculina oblonga* (Lützen and Yamaguchi, 1999)

parasitising *Cyclograpsus intermedius* (Ortmann, 1894) collected at Amakusa, Japan (n = 2) and *Sacculina* sp. parasitising *Nectocarcinus integrifrons* (Latreille, 1825) in Western Port, Australia (n =2) (Table 1).

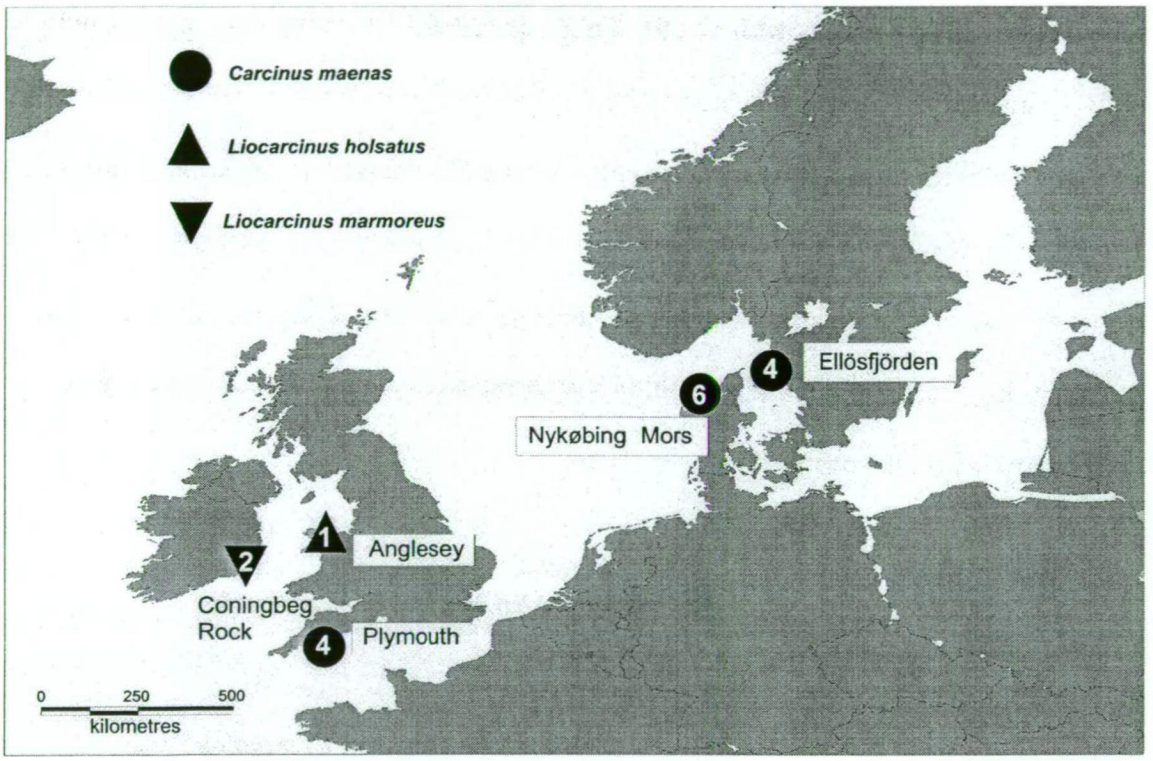


Figure 1. Collection sites for *Sacculina carcini* parasitising three species of crab

Most DNA samples were those previously extracted by Murphy and Goggin (2000) using phenol/chloroform with precipitation from the aqueous solution using ethanol and a CTAB (hexadecyltrimethylammonium bromide) protocol modified according to Grewe *et al.* (1993). This was supplemented with DNA from the six specimens from Nykøbing, Denmark, extracted using the same DNA extraction protocol. All DNA extractions came from specimens preserved in 70% ethanol. Sequence data

from an *S. carcini* parasitising *C. maenas* in Sweden was obtained from Genbank (Accession number AY117692).

The COI region was amplified from all isolates by polymerase chain reaction (PCR) using the primers HCO2198 (5′–TAAACTTCAGGGTGACCAAAAAATCA–3′) and LCOI490 (5′–GGTCAACAAATCATAAAGATATTGG-3′) (Folmer *et al.*, 1994). The DNA was denatured at 94° C for 15 seconds, primers annealed at 50° C for 15 seconds and chains extended at 72° C for 2 minutes, for 35 cycles. The primer set amplified a 652 bp segment of the cytochrome oxidase I mitochondrial gene. The amplified product was confirmed on 1.2 % agarose gels stained with ethidium bromide. Amplified products were purified with QIAquick spin PCR purification kits (QIAGEN INC., Chatsworth). Each PCR product was sequenced in both forward and reverse directions using Big Dye Terminator chemistry and run on an automated sequencer (ABI 3100 DNA capillary sequencer) according to the manufacturer's instructions. After primer sequences were removed, nucleotide sequence data of nominal *S. carcini* were aligned with those of *S. granifera*, *S. oblonga*, *H. lunatas* and the undescribed rhizocephalan from *N. integrifrons* for comparison. Alignment was done using Clustal software. All sequence data were submitted to Genbank (See Table 1 for accession details).

Table 1. Rhizocephalan specimens examined for COI gene sequence data.

Rhizocephalan	Host	Site	Gen Bank Accession Number	Catalogue Number Tasmanian Museum and Art Gallery
Sacculina carcini	*Carcinus maenas*	Nykøbing, Denmark	DQ059766 DQ059767 DQ059768 DQ059769	 G5471 G5472
Sacculina carcini	*Carcinus maenas*	Ellösfjörden, Sweden	DQ059770 DQ059771 DQ059781	G4024 G4027
Sacculina carcini	*Carcinus maenas*	Plymouth, England	DQ059772 DQ059773	G4025
Sacculina carcini	*Liocarcinus marmoreus*	Coningbeg Rock, Ireland	DQ059774	G4026
Sacculina carcini	*Liocarcinus holsatus*	Anglesey, Wales	DQ059775	
Sacculina sp.	*Nectocarciuns integrifrons*	Western Port,Victoria, Australia	DQ059776 DQ059777	G4029
Heterosaccus lunatus	*Charybdis callianassa*	Moreton Bay, Queensland, Australia	DQ059778	
Sacculina granifera	*Portunus pelagicus*	Moreton Bay, Queensland, Australia	DQ059779	
Sacculina oblonga	*Cyclograpsus intermedius*	Amakusa, Japan	DQ059780	G4028

A bootstrap test of phylogeny (10 000 replicates) was run to construct a nearest neighbour tree for all rhizocephalan samples using the Kimura 2-parameter distance measure in the software package Mega version 3.0 (Kumar *et al.,* 2004). The results of a contingency matrix for all nominal *S. carcini* haplotypes (including singletons) by population were compared to the expected chance distribution using a Monte Carlo χ^2test (Zaykin and Pudovkin, 1993). Population comparisons were made by testing the significance of genetic distances between pairwise comparisons of haplotypes for all samples of nominal *S. carcini* permuted 10 000 times using F_{ST} analysis in the software package Arlequin version 2.000 (Schneider *et al.,* 2000).

RESULTS

There were large differences in nucleotide sequences among species. This averaged 25% for the five species compared, and in pairwise comparisons ranged from 15% between *S. carcini* and *Sacculina* sp. to 34% between *S. carcini* and *S. oblonga* (data not shown).

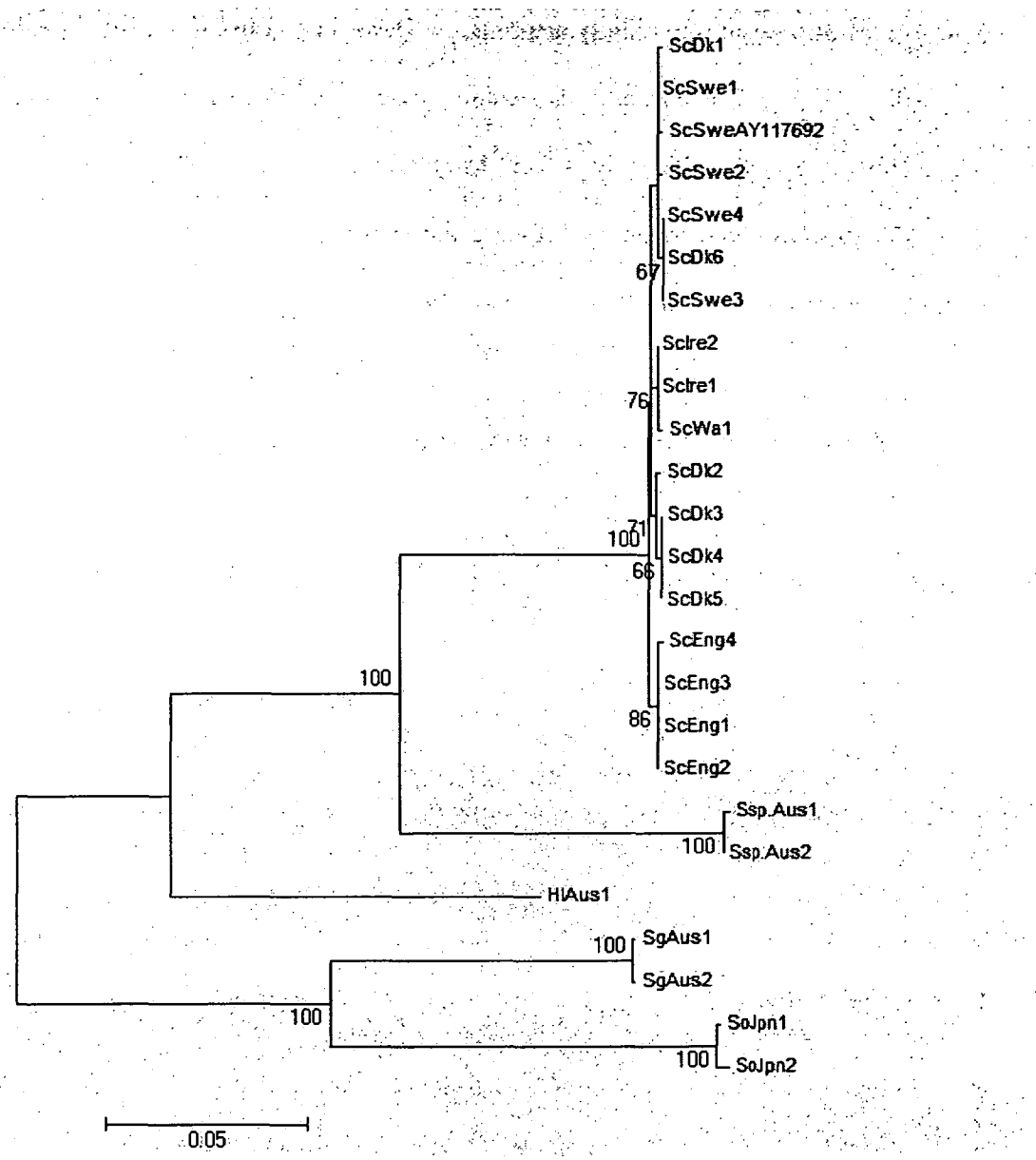
Within the nominal *S. carcini* grouping, there was a 0.6% divergence between *S. carcini* parasitising *C. maenas* from England compared with Denmark and Sweden, and a 0.4% difference between *S. carcini* parasitising *C. maenas* and those parasitising the *Liocarcinus* species collected in Ireland and Wales. The aligned mtDNA sequences revealed 10 haplotypes, A – J (Table 2), suggestive of three geographically separate populations: the Baltic Sea (Sweden and Denmark), the English Channel (Plymouth) and the Irish Sea (Ireland and Wales).

A fixed substitution differentiated Ireland and Wales from other collections at sequence position 500 and a fixed substitution differentiated England from other collections at sequence position 637. The majority of Swedish and Danish specimens had a substitution at sequence position 313, and were also polymorphic at sites 222, 364 and 397. These haplotype similarities and differences were suggestive of three population groupings (Table 2).

Table 2. Aligned COI mtDNA sequences for *S. carcini*. Grey shaded text refers to atypical bases.

Country of Origin & Specimen	*Haplotype*	*Nucleotide Position*	23	169	222	294	313	364	397	500	574	633	637	649	*Host Crab*
Denmark 1	A		A	G	G	t	g	T	C	T	A	t	C	T	*Carcinus maenas*
2	B		A	G	t	C	A	T	C	T	A	C	C	T	
3	C		A	G	t	C	A	c	C	T	A	C	C	T	
4	C		A	G	t	C	A	c	C	T	A	C	C	T	
5	C		A	G	t	C	A	c	C	T	A	C	C	T	
6	D		A	G	G	C	g	T	a	T	A	C	C	T	
Sweden 1	E		A	G	G	C	g	T	C	T	A	C	C	T	*Carcinus maenas*
2	F		A	t	G	C	g	T	C	T	A	C	C	T	
3	D		A	G	G	C	g	T	a	T	A	C	C	T	
4	D		A	G	G	C	g	T	a	T	A	C	C	T	
England 1	G		A	G	G	C	A	T	C	T	A	C	t	a	*Carcinus maenas*
2	G		A	G	G	C	A	T	C	T	A	C	t	a	
3	G		A	G	G	C	A	T	C	T	A	C	t	a	
4	H		A	G	G	C	A	T	C	T	t	C	t	a	
Ireland 1	I		A	G	G	C	A	T	C	c	A	C	C	T	*Liocarcinus marmoreus*
2	I		A	G	G	C	A	T	C	c	A	C	C	T	
Wales 1	J		g	G	G	C	A	T	C	c	A	C	C	T	*Liocarcinus holsatus*

The neighbour-joining tree produced four closely related, but distinct groupings of *S. carcini* specimens that largely mirrored geographic locations (Figure 2). Within the *S. carcini* species, good separation was shown with bootstrap values of 86, 76 and 67% for the geographic groupings of the English Channel, Irish Sea and Baltic Sea, respectively. Bootstrap values showed 100% separation for the four outgroup species. *S. carcini* and *Sacculina sp.* appeared to be more closely related to *H. lunatus* than to their congeners *S. granifera* and *S. oblonga.*



KEY
Sc– *Sacculina carcini* from Denmark, Sweden, Ireland,Wales and England
Ssp.Aus – *Sacculina* sp. Australia
HlAus – *Heterosaccus lunatus* Australia
SgAus – *Sacculina granifera* Australia
SoJpn – *Sacculina oblonga* Japan

Figure 2. Phylogenetic tree of rhizocephalan species based on COI nucleotide analysis. The numbers at the nodes are bootstrap values. The scale bar shows genetic distance.

Monte Carlo χ^2 tests (10 000 runs) of the 10 haplotype distributions across the 5 source countries (Table 2) indicated significant spatial heterogeneity ($\chi^2 = 54.778$, $P <$

0.001). This difference was mainly attributed to Danish and English Channel haplotypes ($\chi^2 = 10$, $P < 0.05$). These findings were confirmed by population pair-wise *Fst* analysis which showed *S. carcini* haplotypes from England were significantly different from the Swedish and Danish haplotypes ($P < 0.05$). *Fst* values were extremely high for all pair-wise comparisons (Table 3).

Table 3. *Fst* Vales for a population pairwise comparison of *Sacculina carcini.*

Population pairwise *Fst* values

	1	2	3	4	5
1	0.00000				
2	0.30644	0.00000			
3	0.58044	0.79167	0.00000		
4	0.34066	0.69399	0.88626	0.00000	
5	0.33333	0.68889	0.88235	1.00000	0.00000

Key

Label	Population name
1	Denmark
2	Sweden
3	England
4	Ireland
5	Wales

DISCUSSION

The results of the COI analysis indicate that there is significant genetic variation among specimens of *S. carcini,* and that a large part of this variability is geographically based. Also, the intraspecific magnitude of divergence for all compared rhizocephalans is of the same order, and the differences among the regions and hosts sampled for nominal *S. carcini* are much smaller than those among *Sacculina* species.

Larger sample sizes of *S. carcini* from the geographical regions presented in this study need to be undertaken in order to assess the level of intraspecific variation more accurately, so that the proposed geographic differences can be stated confidently. Nevertheless, we conclude that all the examined *S. carcini* samples were conspecific, based on an analysis of COI sequence divergence which showed that 99.9% of crustaceans with COI sequence divergence less than 1% are conspecific (Hebert, *et al.,* 2003). Analysis of the COI sequence, therefore, provides considerable potential for resolving, for the first time, the geographic population structure of *S. carcini.* It is also likely to prove useful for analysis of other rhizocephalans. Such analyses have proven difficult based on traditional morphological approaches, due to the much reduced structure of the parasitic adults. However, an analysis of the nuclear encoded rRNA genes (ITS1) and (SSU) used by Murphy and Goggin (2000) showed no variation among individuals of *S. carcini* and consequently revealed no spatial structure. The difference between our results and those in Murphy and Goggin (2000) is consistent with an

expected slower rate of divergence for nuclear, as opposed to mitochondrial, genes (Remegio and Hebert, 2003).

The genetic differences between *S. carcini,* from geographically well separated areas may be due to their very short planktonic dispersal stage (about 6 days) (Høeg and Lützen, 1985, 1995). Although data for most groups are still sparse, there is a general expectation and some supporting evidence that duration of the dispersal phase broadly correlates with degree of population panmixia in marine invertebrates (Weber *et al.*, 2000, although see also Strathmann, 1985; Todd *et al.*, 1988). At only six days, the dispersive phase of *S. carcini* is among the shortest known for a temperate marine invertebrate and this may well explain the localised populations and genetic sub-structuring we have found for *S. carcini.*

We expected greater homogeneity might have resulted from dispersal by mechanisms other than via the planktonic stage, and in particular by the hosts themselves. Our observations of invasive populations of *C. maenas*, in Australia, suggest individuals are highly mobile; as catch rates and size frequency distributions of the crabs change markedly on weekly time scales (C. Proctor pers. comm.). This is consistent with a study of tagged *C. maenas* in lagoons along the coast of Portugal, in which Gomes (1991) reports that, after at most six months freedom, most tagged individuals were re-caught between 1 and 10 km from the point of tagging, and that a few had moved more than 15 km. We expected that the along-coast movement of parasitised crabs over a likely 4–6 year life span would be a significant factor in reducing genetic heterogeneity of *S. carcini*

sub-populations. However, *C. maenas* parasitised by *S. carcini* have been reported to migrate to deeper waters (Rasmussen, 1959) and this effect may explain the genetic heterogeneity of the parasite by restricting coastline migration and maintaining genetic isolation. As a possible means of teasing apart the roles of planktonic and benthic dispersal on population structure, it would be of considerable interest to examine the genetics of parasite populations around islands isolated by relatively deep water. Deep water would act as a barrier, preventing exchange by benthic *C. maenas* and thereby reveal the extent to which gene flow between adjacent populations might be mediated by stepping stone migration. In this regard, it might also be very informative to compare a detailed analysis of the population structure of *S. carcini* with that of *C. maenas* itself (Roman and Palumbi, 2004).

It appears that *H. lunatus* is more closely related to *S. carcini* than the latter is to the congeneric *S. oblonga*, and *S. granifera*, highlighting the difficulty of morphological species identification of the rhizocephala. This finding agrees with Glenner *et al.* (2003), whose phylogenetic analysis of ten species of *Sacculina,* using COI and 18s r gene sequences, suggested that neither the family Sacculinidae nor the genus *Sacculina* is monophyletic.

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CHAPTER 7

GENERAL DISCUSSION

Australia's crab parasite fauna

The number of described species of metazoan parasites found in Australian native crabs is small, amounting to perhaps less than 20 reports in the literature. Many of these reports are from Queensland (Table 1). The records of crab parasites from temperate waters are fewer still, including: a rhizocephalan, *Sacculina* sp.nov. (Chapter 5), parasitising the rough rock crab *Nectocarcinus integrifrons* (Latreille, 1825) (Murphy and Goggin, 2000), two species of Acanthocephalan worm, *Polymorphous (Profilicollis) sphaerocephalus* (Bremser in Rudolphi, 1819)and *Corynosoma stanleyi* (Smales, 1986), parasitising *N. integrifrons* and 5 species of grapsid crab (Pichelin *et al.*, 1998) and observations on trematode metacercariae (Bell, 1988) and a nemertean worm (Bell and Hickman, 1985). The crab parasite survey reported in this thesis has added a possible 12 metazoan parasites to the records. Up to eight of these parasites are likely to be new discoveries and a name, *Sacculina nectocarcini,* has finally been provided for a rhizocephalan, possibly first discovered nearly 120 years ago (Haswell, 1888). There are, undoubtedly, many more parasites to be discovered and described from the crabs of Australia's temperate coastline and the parasites reported in this thesis are likely to represent only a small fraction the parasite fauna. Nevertheless, many of the common intertidal and shallow sub-tidal crabs have been examined providing new information on prevalence and intensity of parasitism, geographic distributions and biological notes on both host and parasite. This research has divulged some of the trophic links which underpin the ecological webs of a marine system which supports much of Australia's recreational and commercial fisheries. The diversity and trophic complexity of

parasite/host interactions reflects the ecological health of this system and the results of this survey will provide benchmark information for future ecological studies.

Table 1. Some metazoan parasites of Australian crabs

Decapod Host	Parasite	Location	Author
Pilumnopeus serratifrons	*Loxothylacus spinulosus* (Rhizocephala)	Sydney, NSW.	Boschma (1928)
Parthenope longimanus	*Sacculina duracina* (Rhizocephala)	Port Molle, Qld	Boschma, 1933
Portunus pelagicus	*Sacculina granifera* (Rhizocephala)	Moreton Bay, Qld	(Boschma 1973)
Matuta granulosa	*Sacculina amplituba* (Rhizocephala)	Moreton Bay, Qld	Phillips (1978)
Charybids truncata	*Heterosaccus multicinencis* (Rhizocephala)		
Charybdis callianassa	*Heterosaccus lunatus* (Rhizocephala)		
Paragrapsus gaimardii	Microphallid trematodes	Bruny Island, Tasmania	Smith (1983)
Bellidilia laevis	*Carcinonemertes* sp. Nemertea	Bruny Island, Margate, Tasmania	Bell & Hickman (1985)
Scylla serrata	*Loxothylacus ihlei* (Rhizocephala)	Northern Australia	Knuckey *et al.* (1995)
Portunus pelagicus	Melogonimus rhodanometra (Digenea)		Bray *et al.* (1995)

Trypanornyhnch tapeworms

Two species of larval trypanorhynch tapeworm, *Trimacracanthus aetobatidis* (Robinson, 1959 and *Dollfusiella martini* (Beveridge, 1990), showed some promise as biological controls. Both species had a wide geographical range with low host specificity, being found in Victoria, Flinders Island and Tasmania and parasitising six species of grapsid crab and two species of portunid crab. Apart from a single case of nematode infection, *T. aetobatidis* and *D. martini* were the only parasites found associated with *Carcinus maenas* (Linnaeus, 1758) in Australian waters. These parasites, suggested the best opportunity for augmentative biological control as they had naturally switched into this novel host. No *C. maenas* found in Australian waters were parasitised by natural enemies from their native range, a common phenomenon for introduced pests (Torchin *et al.*, 2003). Indeed, the number and variety of parasites which have successfully parasitised *C. maenas* in its invaded range throughout the world is low relative to its native distribution (Torchin *et al.*, 2003).

Parasitised *C. maenas*, from Swan Bay, Victoria, showed clear signs of digestive gland pathology at the gross level, which suggested the possibility that trypanorhynch metacestodes might be causing sufficient physiological damage to their host to be considered potential control agents. High intensity infections in *C. maenas* were subsequently shown to cause significant digestive gland pathology which manifested as abnormal digestive enzyme activity (Chapters 3 and 4). Specifically, the parasitised crab mounted an inflammatory reaction typical of the Crustacea, involving haemocyte aggregation around the trypanorhynch plerocercoid which progressed to encapsulation

and melanisation. The digestive gland tubule pathology was characterised by metaplasia of the tubule epithelium and tubule atrophy and necrosis resulting from pressure exerted by the growing plerocercoid. Regression analyses revealed significant negative relationships for the specific activity of trypsin and lipase versus the intensity of infection. Whether digestive enzyme activity was depressed as a direct result of tubule destruction is not clear.

These Trypanorhynch-infected *C. maenas* were found throughout the entire survey range, including Tasmania, which is believed to have the most recent invasion of *C. maenas* in Australia. The Tasmanian invasion is estimated to have occurred about 15 years ago (Thresher *et al.*, 2003). *Carcinus maenas* has quickly become part of the trophic cycle in these newly invaded areas enabling trypanorhynch transmission to become established in a new host. The Rapid entry of *C. maenas* into the trophic cycle of these parasites, suggests that an augmentative approach is likely to succeed, at least in terms of ready transfer of the control agent to the pest. The final hosts for these tapeworms are sharks and rays and are common and widely distributed throughout the *C. maenas* range (Last and Stevens, 1994). *Carcinus maenas* is sympatric with native grapsid crabs and the native, *N. integrifrons* which act as a reservoir of trypanorhynch plerocercoids for continued supply to the trophic cycle. It is interesting to note that only *C. maenas* from the east coast of Tasmania have become parasitised by *T. aetobatidis*, I have never recorded parasitised *C. maenas* from the north coast of Tasmania (Gurney unpubl. data). The lack of parasitised *C. maenas* from sites sampled along the north coast of Tasmania may be due to the physical nature of the sites. None of the northern sites were located on

a large bay or lagoon, most were small river mouths which may be unsuitable habitat for final host rays and sharks to complete the trophic cycle for this parasite.

In terms of the principle of safety, which must underlie biological control efforts, these trypanorhynch tapeworms are part of the native fauna. They have co-evolved with their hosts and represent an intimate association with the eco-system and consequently pose no new threats. The second principle, efficacy of the parasite against the target organism, has been demonstrated, as discussed above, in terms of physical pathology and physiological impairment to the host. However, despite trypanorhynchs fulfilling these two major principles of safety and efficacy, there are potential difficulties with the practical application of these parasites for biological control in the field. To begin with, the trypanorhynch tapeworm lifecycle remains to be elucidated. The trypanorhynch lifecycle is known to include second intermediate crab, fish and mollusc hosts with sharks and rays as final hosts. The first intermediate host is presumed to be a copepod, based on the experimental work of Sakanari and Moser (1985). Completing the lifecycle for these trypanorhynchs is essential for a complete understanding of the trophic links in order to foresee potential indirect consequences for other trophically linked species.
For an augmentative approach to succeed, trypanorhynch eggs would need to be produced or collected for hatching and infection of the first intermediate host, using mass production techniques in a laboratory/hatchery. These techniques have yet be developed. And while Australian native crabs, sharks and rays have co-evolved with the native trypanorhynchs, adding large numbers to the environment could burden non-target hosts with parasite loads they might otherwise not have encountered.

Furthermore, how this parasitism affects the behaviour and survival of parasitised *C. maenas* remains unclear and requires further study. Survival at the population level would involve studies of infected populations of native crabs and *C. maenas* with comparison against uninfected or lightly infected populations. The crab survey included in this thesis has shown uninfected and lightly infected groups of both native and *C. maenas* to exist. These populations could provide the necessary data on fecundity, survival, population density and age structure to design computer simulations to predict the level of biological control trypanorhynch tapeworms might afford.

A complex trophic cycle makes an augmentative approach (simply adding or enhancing parasites to a system) difficult to administer and monitor. The chain of feeding events which are necessary to complete the trypanorhynchs' life cycle is dependent on the variable recruitment of its numerous hosts. A recruitment failure of any single host may translate into reduced parasite production and consequently reduced parasitisation for subsequent hosts. Because the degree of pathology produced by trypaonrhynchs in *C. maenas* is intensity dependent, high parasite loads are required to cause host damage. A single infection will not impair its host. The practical application of these parasites as biological controls for *C. maenas* will be difficult and should not be attempted until their complete life cycles are determined.

Rhizocephala, *Sacculina nectocarcini* sp. nov.

A directly transmitted parasite which has a single host is much simpler to apply and monitor for biological control programs. Wasps and parasitic flies have been the mainstay

of terrestrial insect control and were one of the earliest discoveries leading to the development of entomology and biological control (van Lenteren, 2005). These natural enemies are parasitoids, they have a single host which they directly attack, parasitise and kill and in this way resemble predators (Roberts and Janovy, 1996). Parasitic castrators, a term applied to marine parasites which destroy the gonad of their hosts, effectively act as parasitoids (Kuris, 1974). Instead of killing their infected prey, and thereby preventing the host from reproducing, the parasitic castrator destroys the gonad of its host, allowing the host to survive while preventing reproduction. As a method for biological control, parasitic castrators surpass parasitoids, because the surviving host remains to ensure wasted mating effort with fertile individuals, an effect which further contributes to population control.

The parasitic castrator considered the most promising native control agent for the control of *C. maenas*, was the rhizocephalan *S. nectocarcini* sp. nov., discovered parasitising populations of *N. integrifrons* from Western Port, Victoria, by G. Ruiz in a body of water known to be invaded by *C. maenas*. Very little was known about this parasite. It may have first been detected in Port Jackson in 1888 (Haswell, 1888) but since that first report, it has not been mentioned in the scientific literature until its recent discovery (Murphy and Goggin, 2000). The main distinguishing features are the structure of the mantle papillae, the retinaculae and the male receptacles. The combination of characters seen in this species is not known from any described rhizocephalan and these characters, in combination with an analysis of the mitochondrial DNA (CO1 region) (Chapter 6) and

ribosomal RNA (ITS1 region) (Murphy and Goggin, 2000) reveal it to be a separate species (Chapter 5).

Nectocarcinus integrifrons, a native host of *S. nectocarcini* sp. nov., is the nearest Australian relative of *C. maenas*, belonging to the family Carcininae (cf. Poore, 2004). The host specificity of sacculinids is variable, with some showing extreme single host specificity and others multiple host affinities (Høeg and Lützen, 1995). *Carcinus maenas* for example, has been reported to parasitise two different orders of crab: Pirimelidae and Portunidae (cf. Høeg and Lützen, 1985). If the Australian sacculinid were to become a control agent against *C. maenas,* its host specificity must be sufficiently lax to enable it to switch hosts at the family level. Although collections of 143 *C. maenas* from Western Port failed to find any rhizocephalan-infected crabs, the possibility of this parasite switching to *C. maenas* hosts remained, as this parasite has been shown to have a patchy geographic distribution on its native host and searches for infected *C. maenas* may have simply missed infected populations.

Laboratory trials were subsequently performed to test cross-infectivity of *S. nectocarcini* sp. nov., with *C. maenas*. Unfortunately difficulties rearing infective *S. nectocarcini* sp. nov., larvae were encountered and host specificity for this parasite could not be determined. Molecular methods of testing rhizocephalan infectivity have been devised by (Thresher *et al.* (2000) and Goddard *et al.,* in press. These methods are capable of detecting infective primordial rhizocephalan cells which are destroyed by the immune system of an incompatible host. Such a method might reveal whether *C. maenas* from

Western Port, were at least being infected by cypris larvae from the sacculinid parasitising the sympatric *N. integrifrons*.

The molecular analysis of COI mitochondrial DNA from four species of *Sacculina* (Chapter 6) showed the greatest homology (85%) occurred between the sacculinid parasitising *S. nectocarcini* sp. Nov., and *S. carcini.* It may be hypothesised that the closer the genetic relatedness of two parasitic species, the greater the chance for host switching between those related species. Rhizocephalan barnacles are intimately connected both physically and physiologically to their hosts, but do not elicit an immune response from their natural hosts, suggesting histocompatibility derived from parasite and host co-evolution. It may be that closely related rhizocephalan species will express proteins of more similar structure than distantly related congeners. As a result, closely related rhizocephalans are less antigenic to their close relative's natural host, and this may greatly increase the chance for host switching. Supporting evidence for this hypothesis may come from the host side of the host/parasite relationship. Poulin *et al.* (2000) have proposed that parasite species may be more successful at parasitising hosts with low levels of genetic variation. In other words, a higher rate of parasite species are accumulated in genetically homogenous host species than related but genetically more variable species.

Classical Biological Control: Potential control agents against *C. maenas*

Classical biological control introduces a non-native control agent from the pest's native range to control it in its new invaded range (Secord, 2003). *Sacculina carcini* (Thompson,

1836) has been considered as a possible classical biological control agent against *C. maenas* (Lafferty and Kuris, 1996, Høeg *et al.,* 1997, Thresher *et al.,* 2000, Goddard *et al.,* in press). So far *S. carcini* has not passed the principle of safety in terms of host specificity. *Sacculina carcini* has been reported in both portunids and one species of pirimelids in its native range (Høeg and Lützen, 1985). Murphy and Goggin (2000) found *S. carcini* to parasitise three species Portunid, including *C. maenas,* based on a molecular analysis of nucleotide sequence data from ribosomal RNA, and the analysis of mitochondrial CO1 DNA (Chapter 6) confirmed their findings. The use of *S. carcini* as a classical biological control agent requires careful consideration after recent host specificity testing determined the parasite to be lethal in laboratory-infected native Californian crabs (Goddard *et al.,* in press).

However, under natural conditions, mortality in novel crab hosts parasitised by *S. carcini,* may not be as high as in laboratory tests due to the mitigating effects of encounter and compatibility filters (Kuris *et al.,* in prep). The complex life history of rhizocephalans in general, and *S. carcini* in particular, means that infective *S. carcini* female cypris larvae could be mass released into a site invaded by *C. maenas* to test host specificity in natural conditions, without long term consequences for native crabs. The female cypris larvae settle on their host and inject a vermiform body which ramifies to produce an interna which, in turn, induces behavioural, morphological and physiological changes leading to castration (Glenner *et al.,* 2000, Høeg and Lützen, 1995). When the rhizocephalan develops towards sexual maturity, it produces an external brood chamber (externa) which erupts through the abdominal cuticle of the host crab. The externa has to be implanted by

a male cypris larva to ensure its survival and reproduction (Høeg and Lützen, 1985). Because only female larvae are initially released en masse, there are no male larvae in the environment to fertilise the externa. The unfertilised externae of all infected crabs will die and so too will the infected hosts (Høeg *et al.*, 1997). Field studies, from such a release, would provide invaluable information on prevalence of infection and mortality for targeted *C. maenas* as well as native crabs, which may be susceptible to parasitisation. The results of such an experiment could lead to one of three possible outcomes: 1 classical biological control may be deemed safe enough for the release of both male and female cypris larvae to establish a self sustaining parasite population. 2 carefully regulated mass releases of only female cypris larvae could be used to control *C. maenas* because of limited infection of native crabs. 3. Host specificity of *S. carcini* is too lax for it to be safely released in any form.

Other possible candidates for biological control of *C. maenas*

Dinoflagellates, and ciliates have been suggested as possible biological control agents for *C.maenas,* however, these natural enemies have been deemed insufficiently host specific or virulent to be effective (Goggin, 1997). Furthermore, being micro-organisms they are too likely to evolve to exploit new hosts (Secord, 2003).

Other possible candidates for classical control of *C. maenas* include, for example, the recently re-discovered *Fecampia erytrhocephala* (Giard, 1896), a flat worm parasitoid (cf. Kuris *et al.*, 2002) and the entoniscid isopod, *Portunion maenadis* (cf. Searle and Crompton, 1995). Nemertean worms in the genus *Carcinonemertes* are symbiotic egg

predators on a wide range of crab hosts, including *C. maenas*. However, their lack of host specificity does not make them good biological control candidates (Comely and Ansell, 1989, Torchin *et al.,* 1996, Goggin, 1997).

Augmentative biological control might be applied to *C. maenas*, using an undescribed species of carcinonemertean worm infecting the Australian native crab *Bellidilia laevis* (Bell, 1855) (cf. Bell and Hickman, 1985). *Bellidelia laevis* is sympatric with *C. maenas* and offers host switching possibilities, however the host specifity for this parasite is unknown and needs to be resolved. Similarly, the undescribed entoniscid isopod found in *N. integrifrons* may serve as an augmentative biological control agent providing it is able to host switch to *C. maenas*. This parasite had a wide geographic distribution being found in both Victoria and Tasmania. The taxonomic relatedness of its hosts, *N. integrifrons* and *C. maenas,* likely confers greater host switching success for this parasite than for the carcinonemertean, described above, which shares ecological overlap between the hosts *B. laevis* and *C. maenas*.

Unless *C. maenas* establishes as a pest of significant economic consequence for aquaculture or the fishing industry, the use of exotic control agents for biological control is difficult to justify. Many of the possible exotic control agents are not sufficiently host specific to be used with confidence. The management of invasive pests is increasingly being developed to integrate many different methods to achieve control and is referred to as integrated pest management (IPM). These methods include, for example, physical removal or destruction of pests, public education and participation, improved border

control and quarantine and selective chemical pesticides along with the range of possible biological control methods. In order to apply the most effective combination of control methods for any particular pest, there needs to be a clear understanding of the pests' biology and impact upon the ecology of its invaded range. It has been noted that some populations of introduced species experience rapid declines which are difficult to explain and may lead to extinction (Simberloff and Gibbons, 2004). *Carcinus maenas* is continuing to extend its Tasmanian range southwards of the Derwent estuary (Gurney unpubl. data) but its numbers are low relative to those reported in the summer of 1996/97 in Georges Bay, Tasmania (Thresher *et al.* 2003). It is conceivable that *C. maenas* is experiencing population stasis which requires further investigation in order to make better informed decisions about future methods of control.

In addition to sound scientific knowledge, the successful application of IPM is also dependent upon the risks and benefits as perceived by society. Low risk Management methods such as physical removal are likely to have favourable public response but in many cases will have limited effect, particularly for large scale incursions. Conversely, higher risk methods with greater chance of success, such as the introduction of an exotic virus, may be considered too risky to the environment or human health and will therefore be deemed socially and politically unacceptable (Thresher and Kuris, 2004). The proposed use of exotic species such as *S. carcini* to control *C. maenas* in invaded ranges has been met with some resistance (Thresher, 1997, Murphy and Goggin, 2000, Secord, 2003). However, augmentative biological control, using the native species of parasites found in Australia's shore crabs, offers the potential for an effective and publicly

acceptable form of control for *C. maenas*. According to Secord (2003) 'augmentative biocontrol ... should be given high priority in marine environments'. While acknowledging the practical difficulties involved, the process of host switching from native crabs to *C. maenas* has already occurred, both in Australia (trypanorhynch tapeworms) and in other invaded regions of the world (Torchin *et al.*, 2003). Subtle manipulations (mass rearing, seasonal releases) of potential native enemies, described above, may produce a politically and socially acceptable array of control agents with some chance of success when used in IPM of *C. maenas*.

Findings and conclusions

Many new parasites have been found in crabs from Australian temperate waters. These discoveries are a small step toward better understanding the parasite fauna and trophic relationships amongst this group of animals and provides a temporal record of parasite diversity in a temperate marine environment. Such a record will act as a benchmark for future studies of parasite ecology in temperate marine intertidal and shallow sub-tidal zones and may contribute to a better understanding of how an invasive species integrates into a new environment over time. Future parasite surveys of *C. maenas* in Australian waters may reveal a greater guild of host switching parasites including, for example, the trematodes and acanthocephalans which have transferred into *C. maenas* in other invaded areas of the world (Torchin *et al.*, 2001). Bacterial, viral and protistan pathogens were not examined in this survey. Presporogonic stages of microsporidea were observed in the hepatopancreas of both the native crab *N. integrifrons* and *C. maenas* and warrant further investigation.

A new species of rhizocephalan has been described, *Sacculina* sp. nov. (Chapter 5), after remaining forgotten to science for approximately 120 years, and adds to the short list of described rhizocephalans from Australia. This rhizocephalan will offer opportunities to study the effects of parasitism on native crabs at the population level and will provide a model for host and parasite interactions of portunids and sacculinids which could in turn be directed towards future biological control of *C. maenas*. The unsuccessful attempts to produce and rear cypris larvae from this parasite in the laboratory were a major limitation to gaining a better understanding of host specificity through infection trials of other crab species. The laboratory rearing systems used successfully for other cyprids have been simple, static water systems (Glenner and Werner, 1998, Thresher *et al.*, 2000) A similar simple system was used to successfully rear cyprid larvae from an undescribed rhizocephalan parasitising *B. laevis* (Chapter 2), however, this system did support cyprid larvae from the rhizocpehalan parasitising *N. integrifrons* (Appendix 1).

Intermediate crab hosts for the trypanorhynchs, *T. aetobatidis* and *D.a martini* have now been determined. The final hosts for these parasites include the gummy shark, *Mustelus antarcticus* (Günther, 1870) (Beveridge pers. comm.) which is a major component of the southern Australian shark fishery (Last and Stevens, 1994). The discovery of the intermediate crab hosts establishes direct trophic links which may provide important management information for this commercially valued fishery.

The effects of high intensity trypanorhynch infections of *C. maenas* have been shown to produce severe digestive gland pathology, possibly resulting in impaired physiological

function. Apart from nematode infections of a single specimen of *C. maenas* (Chapter 2) and a nematode symbiont of *C. maenas* egg masses (K. Culbert pers. comm.) *T. aetobatidis* and *D. martini* are the only metazoan parasites discovered, so far, to have switched from native crabs into *C. maenas*. Their ability to inflict damage to their *C. maenas* hosts shows some promise for their continued consideration as biological control agents and opens the way for future studies to determine whether trypanorhynchs alter the behaviour of their hosts to ensure trophic transmission to sharks and rays.

The ability of *S. carcini* to parasitise three species of portunid host has been re-confirmed (Chapter 6), demonstrating the lax host specificity of this parasite. The introduction of *S. carcini* as a biological control agent for *C. maenas* must, therefore, be approached with caution.

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APPENDICES

Appendix 1 — Testing the host specificity of a parasitic barnacle, *Sacculina* sp., which naturally infects *Nectocarcinus integrifrons*.

Appendix 2 — Morphological and biological notes on *Polymorphous* (*Profillicollis*) *sphaerocephalus* and *Corynosoma stanleyi* (Polymorphidae: Acanthocephala).

Appendix 3 — Invasion dynamics of the European shore crab, *Carcinus maenas*, in Australia.

TESTING THE HOST SPECIFICITY OF A PARASITIC BARNACLE, *SACCULINA* SP., WHICH NATURALLY INFECTS *NECTOCARCINUS INTEGRIFRONS.*

INTRODUCTION

The parasitic barnacle, *Sacculina carcini*, is a natural enemy of the European green crab, *Carcinus maenas.* This barnacle has been proposed as a possible control agent for invasive European green crab populations, based on the parasite's ability to adversely alter the physiology, growth, and behaviour of its host (Lafferty and Kuris, 1996).

A biological control agent's effectiveness and safety must be demonstrated before it can be released into the field (Lafferty and Kuris, 1996). Safety primarily relates to a control agent's degree of host specificity. The perfect control agent should be highly host specific, only attacking its target, the natural host. A number of experiments have been performed to determine the host specificity and effectiveness of *S. carcini* as a potential control agent for *C. maenas* (Thresher *et al.*, 2000, Goddard, *et al.*, 2005). In these experiments non-target crabs, endemic to the areas invaded by the green crab, were exposed to the infective female cypris larvae of *S. carcini* to determine the risk of infection to non-target species.

C. maenas is a well established exotic found in many shallow bays and estuaries of Australia's temperate coastline, extending from South Australia to Victoria, Tasmania and New South Wales. The use of *S. carcini* as a potential biological control agent against *C. maenas* in Australian waters has been considered possible provided the safety of introducing the agent is stringently evaluated (Høeg *et al.*, 1997). However, the discovery of a Sacculinid which infected *Nectocarcinus integrifrons*, a temperate water native Australian crab sympatric with *C. maenas,* suggested the possibility of immediately testing a sacculinid, endemic to Australian waters, against *C. maenas* without the need for rigorous safety testing.

Instead of applying the classical approach of using the natural enemy, *S. carcini*, against *C. maenas*, it may be possible to use a non-natural enemy, the sacculinid which infects the Australian native crab *N. integrifrons*. The benefit of this approach is that native Australian crabs would not be exposed to an introduced control agent. For this approach to have some effect, the host specificity of the Australian sacculinid needs to be low in order to switch hosts from *N. integrifrons* to *C. maenas*. While there are no Australian crabs closely related to *C. maenas*, *N. integrifrons* is related by the sub-family Carcininae (Poore, 2004). This relationship may be close enough to allow for the Australian sacculinid to switch hosts. It is acknowledged that based on experience gained from agricultural insect pests, potential control agents from the new invaded region do not transfer to pests at a rate which produces significant control and this pattern has been observed for invasive green crabs (Kuris, 1997). Nevertheless, the possibility of using a native control agent against the invasive green crab has been investigated and this approach was first attempted using the native Australian sacculinid, *Heterosaccus lunatus*, against *C. maeans* (Thresher *et al.*, 2000).

Three cross infection experiments were run to test whether the Sacculinid from *N.integrifrons* would infect *C. maenas*.

Experiment 1. Attempts were made to collect cypris larvae from a single infected crab in order to determine cypris sex ratios elicited from an expected bimodal size distribution. Cypris larvae were also to be collected and fixed for future description. The aim of *experiments 2* and *3* was to hatch nauplius larvae from infected *N. integrifrons* in the presence of co-habiting *Carcinus maenas* in an attempt to cross-infect *C. maenas* with the subsequent infective cypris larval stage.

MATERIALS AND METHODS

Experiment 1. A single *N. integrifrons* with an externa containing developing eggs was placed into a 40 litre aquarium containing static sea water. Water temperature was maintained at 13°C and salinity was 33 ppt. The crab was not fed for 6 days to eliminate water pollution from uneaten food and to keep ammonia and nitrite levels to a minimum. The externa was checked daily to monitor egg develop. A 50% water exchange was performed on the 4th day.

Experiment 2. Sixteen *N. integrifrons* were placed into two separate 40 litre aquaria with conditioned under-gravel biological filters. Water temperature was maintained at 17°C and salinity was 33 ppt. The crabs were arbitrarily divided by size into two groups of 8 large and 8 small crabs to reduce intraspecific aggression. Two crabs, one from each group, had mature externae. The infection status of the remaining 14 crabs was unknown because there were no externae at the time of collection. Initially a donor crab with an externa containing eyed larvae was transferred to bucket containing 4 litres of gently aerated seawater. The intention was to collect hatched nauplius larvae from the bucket and rear them to the infective cypris stage. The cyprid larvae would then be introduced to recipient crabs in a 10 litre aquarium. This method was abandoned when the condition of the externa deteriorated rapidly in the bucket. Instead donor crabs with developing larvae were transferred to a 60 l aquarium, of the same temperature and salinity, and left to co-habit with 13 (recipient) *C. maenas*, [12 mm – 20 mm carapace width (CW)] (Table 1) and 1 (recipient) *N. integrifrons,* presumed to be unparasitised due to it being collected from a Tasmanian population not known to be infected with rhizocephala. This procedure was designed to determine whether resulting cypris larvae would cross-infect *C. maenas*. Two cross infections were attempted. The Experiment ran for 140 days from 31/10/97 – 9/3/98.

Table 1. Size of recipient green crabs

Sex	Carapace Width mm (CW)	Abdominal Width mm (AW)
male	18	2.5
male	21	3
male	18	2.8
male	20	3.5
male	16	2
male	20	3.2
male	17	2.3
male	12	2
female	17	4.5
female	21	4.5
female	19	4
female	20	4.2
female	18	3.5

Experiment 3. Eighteen *N. integrifrons* were used for cross-infection trials under the same tank conditions as in the second experiment. Fourteen of these crabs had externae. As the larvae developed in the externa, the infected *N. integrifrons* donor crabs were transferred to a tank containing 3 small *C. maenas,* (< 30 mm carapace width) recipient crabs and a presumed uninfected *N. integrifrons* recipient crab. The *N. integrfrons* donor crabs were left to co-habit with *C. maenas* recipient crabs in an attempt to cross-infect *C. maenas* with cypris larvae. The Experiment ran for 25 days from 3/4/98 – 28/4/98.

RESULTS

Experiment 1. The developing eggs within the externa developed eyespots but died before release. White fluid exuded from the mantle cavity. Oil droplets, presumed to be the remains of atretic eggs, were clearly observed through the cuticle of the externa.

Experiment 2. Only 2 of the 16 crabs developed externa. Each externa produced two broods but all broods failed to produce free swimming larvae. Both host crabs died shortly after their respective externa died. No externa developed from the remaining 14 crabs. All 14 crabs without an externa died over the course of the experiment. At least five of these crabs had a well developed interna. The remaining 9 crabs had no interna or no obvious interna. One crab died over a weekend and was too putrified for internal examination.

Experiment 3. Over the course of the experiment 10 of the donor crabs died within the first 15 days. Of these 10 crabs, 8 had an externa but released no larvae. Of the remaining 6 crabs with an externa, 4 crabs with a ripening externa were co-habited with green crabs. All 4 donor crabs died within 7 to 13 days of the cross infection trial and none of the externae from these crabs released free swimming larvae (Table 2).

Table 2. Results for experiments 1,2 and 3.

Experiment	Duration (days)	*N. integrifrons* with Externa	Nauplius larvae Released from Externae
1	19	1	0
2	140	2	0
3	25	14	0

The same pattern of larval degeneration and death was observed for all externae. The reproductive cycle would begin with eggs produced in a firm yellow/brown externa. Eyed larvae took 3–4 weeks to develop in the externa but frequently died at an earlier stage of egg development. The dying eggs decayed within the externa releasing small oil droplets which were visible through the externa cuticle. The general appearance of the externa changed from a firm yellow/brown body to a flaccid, brown blotched body. A creamy white coloured discharge of dead eggs or larvae would exude from the mantle opening. On two occasions the externa recovered after aborting the clutch to produce a second brood.

DISCUSSION

Despite three separate attempts, no viable infective cypris larvae were produced for cross infection of green crabs. Externae developed to one of three stages: 1. externa appeared healthy with eggs but the host and parasite died soon after introduction to the aquarium. 2. A healthy externa would swell in size and produce eggs which died during development and would putrify within the externa and both the externa and host would subsequently die. 3. A healthy externa would produce eyed larvae which failed survive and were aborted from the externa.

Larvae from *S. carcini* take 5–6 days to develop to their infective stage and can take up to a further 3 days to settle upon *C. maenas*, their host (Hoeg *et al.*, 1997). On two occasions larvae were released over a weekend but no larvae were observed the following Monday, in either the water column or attached to donor or recipient crabs. On these occasions it is likely that the externa aborted larvae before their full term and expelled them into the aquarium where they rapidly decayed before detection.

Ammonia and nitrite conditions were kept at 0.1 mg/L or below. All green crabs survived the same water conditions for the duration of the experiment. Unlike green crabs, *N. integrifrons* is a delicate crab which is difficult to transport and maintain in an aquarium. Captive uninfected *N. integrifrons* could not be kept for longer than 3 months (Gurney,

unpubl. data). Providing an adequate diet may have been one of the difficulties of keeping this crab. *N. integrifrons* feeds on the living fronds of seagrass, *Posidonia australis* (Klumpp and Nichols, 1983) and the crushed mussel diet, a diet which sustains green crabs for 1 – 2 years in an aquarium (Gurney, unpubl. data), may have been nutritionally deficient. Poor nutrition coupled with infection of an invasive and intimately connected parasite, such as the Rhizocephala, may have been the cause of poor survival for both *N. integrifrons* and its parasite.

While ammonia and nitrite levels were periodically checked and water exchanges performed, tank conditions probably contributed to poor survival. Ideally, infected crabs would have been kept in tanks with flow through water systems and placed into smaller 3 – 4 litre aquaria with recirculating water systems, just prior to larval release (Glenner and Werner, 1998., Thresher *et. al.*, 2000).

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